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RESPONSE OF STEVIA (*STEVIA REBAUDIANA* L.) TO BIOGAS FERTILIZER AND NATURAL EXTRACTS UNDER SOUTH SINAI CONDITIONS

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ABSTRACT: Two field experiments were conducted in 2019 and 2020 seasons at Desert Research Center, Mataria, Cairo, Egypt, and its experimental farm at Ras Sudr Experimental Station in South Sinai, in cooperation with the Department of Horticulture, Faculty of Agriculture Benha University, to study the effect of biogas slurry fertilizer and natural extracts on biomass, chemical constituents, and quality of *Stevia rebaudiana* L. plant. Results showed that biogas fertilizer along with the different natural extracts exerted a significant influence on yield, chemical constituents, stevioside and rebaudioside A., of stevia. The highest values in most of the parameters i.e. shoot fresh and dry weights biomass, leaves fresh and dry weights biomass, N, P, K and pigments in the first cut by recommended dose of chemical fertilizer (T_1) combining with garlic as drench addition, Yucca extract as a foliar spray or Azolla extract as drench addition (M_4, M_5 or M_2, respectively). On the other hand, the second cut took the same line as the first cut but with 50% NPK + 5000 kg h^{-1} (T_3) as either garlic extract or Azolla extract as drench addition ($T_3 \times M_4$ or $T_3 \times M_2$) as registered the highest values of parameters mention before in most cases. As for the highest values of stevioside and total stevioside content were recorded by 50% NPK + 3000 kg h^{-1} combining with Azolla aqueous extract as drench addition ($T_4 \times M_2$). Rebaudioside A., registered by combination of the recommended dose of biogas fertilizer and yucca aqueous extract as a foliar spray ($T_2 \times M_5$). Conclusively, the use of half (50%) of the recommended dose of chemical fertilizer with its equivalent of the recommended nitrogen dose and replaced with biogas fertilizer, in addition to the use of both extracts of Azolla and garlic as a drench addition, led to reducing nitrate accumulation as a problem of the use of chemical fertilizers on the human health and economic damages.

Key words: *Stevia rebaudiana*, organic fertilizer, natural extracts, Stevioside, rebaudioside A., biogas fertilizer, Azolla extract, garlic extract, yucca extract.

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INTRODUCTION

Stevia (*Stevia rebaudiana* L.) is a green herb plant that belongs to family Asteraceae its leaves contain several chemical substances called glycosides in addition, it contains volatile oils (Hossain *et al*., 2017). These glycosides have a sweet taste but do not contain any calories. The main glycoside is known as stevioside, it is 300-400 times sweeter than sucrose (Singh *et al*., 2019; Talevi, 2021). Chemical fertilizers have been indispensable in agriculture, but they are currently prohibitively costly and, in some cases, unavailable on time (Naguib, 2011). The combined use of organic and chemical fertilizers can minimize dependency on costly chemical inputs. To maintain high crop yields while maintaining soil fertility, it is crucial to produce an optimal fertilizer and manure combination in the crop (Roba, 2018).

Biogas slurry (BGS) is a byproduct of biogas processing using cow dung. Biogas slurry is an excellent source of plant nutrients and can enhance soil properties (Garg *et al*., 2005). According to (Khan *et al*., 2015), biogas slurry contains many rich and nutritive elements such as N, P, K, and trace elements (Zn, Fe, Cu, Ni, and so on). Farmers must use chemical fertilizer to increase crop yield, however, if only mineral fertilizers are added to the soil without the addition of organic manure, land productivity would decrease, (Sonklien *et al*., 2020)

Farmers and researchers are currently focusing heavily on bio stimulants to increase agricultural sustainability; nevertheless, additional natural products such as (yucca, Azolla, and garlic extracts), should be examined, studied, and appraised to improve agricultural sustainability, namely crop quality and quantity. Several prior research found that plant extracts affected hormones, organic acids (Abou Chehade *et al*., 2018), and polyphenols (Lucini *et al.*, 2018).

The garlic extract is made from the sap of the garlic bulb *Allium sativum* (L.), which belongs to the Liliaceae family. It is characterized by a high concentration of Sulphur-containing amino acids, such as cysteine and methionine (Synge, 1970). Garlic also includes volatile oil, allicin, alliin, sugar, iodine, and vitamins are among the products used (Al Mayahi and Fayadh, 2015). According to Abou Hussein *et al*. (1975 a) and Abou Hussein *et al*. (1975 b). Garlic extract has a wide range of effects due to its hormonal origin, which plays an important role in cell lateral extension and elongation. Concerning the garlic acid effect, treatment of *Majorana hortensis* and *Salvia officinalis* with garlic extract at 50 or 100 percent concentration increased fresh and dry weights, photosynthetic pigments of chlorophylls a and b, and total soluble carbohydrates content in the first and second cut, as well as total oil content (El-Rokiek *et al*., 2019)

Azolla has a high nitrogen content and has been used as an environmentally safe fertilizer for wetland rice production, as well as for its ability to retain significant quantities of nutrients (Enwall *et al*., 2005). It is one of the additional types of fertilizer used in addition to other bio-fertilizers. (Rabie *et al*., 2020) on *Matricaria chamomilla* concluded that the addition of compost and 50% of chemical fertilizer to Azolla extract at 50% as foliar (spray) have a positive effect on chemical constituents except for nitrate and nitrite concentrations.

Yucca schidigera, a plant that grows in some of the harshest conditions. It is a natural extract of yucca which is abundant in steroidal saponins, as well as other stress-relieving compounds and antioxidants. When added to the soil with liquid starters, Yucca extract acts as a wetting agent, minimizing the formation of dry spots (Piacente *et al*., 2005). So, the aim of this study is to improve the yield, chemical constituents and quality of *Stevia rebaudiana* L. plant by using biogas slurry as organic fertilizer and natural extracts under the conditions of sandy soil in south Sinai.

MATERIALS AND METHODS

Two field experiments were conducted in 2019 and 2020 seasons at Desert Research Center, Mataria, Cairo Egypt, and its Experimental Farm at Ras Sudr Experimental Station in South Sinai, to study the effect of biogas fertilizer and natural extracts on the biomass, chemical composition, and quality of the *Stevia rebaudiana* L. plant. The experimental site is located in arid conditions; The annual rainfall of 15 mm rainfall ranges to 30 mm and occurs during a short period from November to March. The average annual relative humidity is 57.2%, and it represents the highest temperature during the year that may reach 43 °C, the average maximum temperature during the year is 27.5 °C and the average minimum temperature is 15.2 °C during the two successive seasons of 2018/19 and 2020. The physical and chemical properties of the soil sample were determined, according to (Rathje, 1959), and are shown in Table (1,2). Drip irrigation lines were installed within 20 cm between drippers with an average of 4L/h. Underground water was used as an irrigation source analyses of the irrigation water samples were determined according to (Rayment and Lyons, 2010) and their properties are shown in Table (3).

Seeds of Stevia were gained from Stevia Farm Egypt Co., Cairo, Egypt, which were harvested in early November of the previous season. The seeds were sown on January 1st in a mixture of 3:1 v/v peat moss: vermiculite. The seed trays were placed in polyethylene greenhouses and irrigated thoroughly until germination, then the seedlings trays were transferred to saran (63% shade) until reached acceptable size (7 cm height with 2-3 pairs of true leaves). The seedlings were cultivated in the field on 15th April. The experimental plot was 5.85 m^2 (20 x 70 cm), all plots included eight rows each row was 70 cm apart and twenty meters in length. The treatments were arranged in a split-plot design with three replicates.

The main plots were occupied by biogas and chemical fertilizers levels treatment (T) in randomly distributed, while the sub-plot was occupied by natural extracts of Azolla, garlic and yucca as foliar and drench application treatments (M) in randomly arranged Main plots (T_1, T_2, T_3, T_4):

Main Plot:

T_1: 100% recommended dose (RD) of NPK (100:50:50 Kg/ha).

T_2: 100% recommended dose of biogas slurry (BGS) (6000 kg/ha).

T_3: 50% recommended dose (RD) of NPK + biogas slurry (BGS) (5000 kg/ha).

T_4: 50% recommended dose (RD) of NPK + biogas slurry (BGS) (3000 kg /ha).

Table 1. The chemical properties of the experimental soil.

pH	E.C. (ds/cm)	O.M. (%)	Cations (meq/l)				Anions (meq/l)			
			Ca^{++}	Mg^{++}	Na^{+}	K^{+}	HCO_3^-	SO_4^{--}	Cl^-	CO_3^{--}
7.95	3.14	0.7	3.44	8.95	3.43	1.8	3.22	3.48	10.92	0
			Available macronutrients (mg/kg)				**Available micronutrients (mg/kg)**			
			N	**P**	**K**	**Ca**	**Fe**	**Mn**	**Cu**	**Zn**
			60	27.24	73	306	94.92	12.92	17.4	3.88

Table 2. Physical properties of the experimental soil.

Coarse Sand (%)	Fine Sand (%)	Silt (%)	Clay (%)	Soil texture
46	50	3	1	sandy

Table 3. Water analysis of the irrigation water.

pH	E.C. (ds/cm)	Cations (meq/l)				Anions (meq/l)			
		Ca^{++}	Mg^{++}	Na^{+}	K^{+}	CO_3^{--}	HCO_3^-	SO_4^{--}	Cl^-
7.95	0.8	3.09	0.8	0.93	0.48	0	2.16	1.57	1.90

Sub Plot:

M_1: Azolla aqueous extract (50%) as a foliar spray.

M_2: Azolla aqueous extract (50%) as drench addition.

M_3: Garlic aqueous extract (10%) as a foliar spray.

M_4: Garlic aqueous extract (10%) as drench addition.

M_5: Yucca aqueous extract (0.3 g/l) as a foliar spray.

M_6: Yucca aqueous extract (0.3 g/l) as drench addition.

M_7: Distilled water as a foliar spray.

M_8: Distilled water as drench addition.

The experiment was conducted for six months from April to October for each season.

Biogas slurry fertilizer (BGS):

Biogas doses were added during the site and row preparation in assigned experimental units nitrogen, phosphates, and potassium fertilizers as ammonium nitrate (33% N) at the rate of 100 kg/hectare, Calcium superphosphate (15.5% P_2O_5) at the rate of 50 kg/hectare and potassium sulphate (48% K_2O) at the rate of 50 kg/hectare, respectively were added to assigned experimental units. Half of the nitrogen dose and the entire doses of phosphorus and potassium were added as a basal dose during assigned experimental rows preparation and the remaining nitrogen was applied after second cut, other treatments of natural aqueous extracts were applied as a foliar spray as well as drench (dressing application) in the early morning starting 15 days after transplanting seedlings at one-month Intervals using biofilm 1 g l^{-1} as a wetting agent and hand pump sprayer and nozzle in both Azolla and Garlic aqueous extracts .

The biogas slurry fertilizer (BGS) used in the experiment was obtained from the Land and Water Research Institute, Agriculture Research Center. The chemical characteristics of biogas slurry are given in Table (4).

Natural Extracts preparation:

Garlic extract:

The garlic extract (*Allium sativum*) was prepared according to (Elzaawely *et al*., 2018) as 100 g of freshly grown cloves of garlic were brought and were macerated in 100 ml of tap water in a tightly stoppered beaker and kept in a freezer for 24 hours at (20 ºC). The macerate was then thawed by allowed to melt at room temperature (25 ºC). The melted mixture was blended in a blender for 5 minutes, then the blended macerate was frozen and melted twice. The aqueous macerate extract was kept in the refrigerator (1 ºC) as crude extract for 10 hours and then the blended mixture was filtered through a double layer of cheesecloth. The obtained filtrates were completed by distilled water to a final volume of 1 liter. The chemical constituents of aqueous extract were shown in Table (5).

Azolla extract:

Azolla was obtained from the Land and Water Research Institute, Agriculture Research Center (Table, 6). Azolla extract was extracted from fresh plant parts of Azolla depending on the extraction method according to (Wilson and Al-Hamdani, 1997) with slight modification. Firstly, washing the fresh plant part with tap water, followed by distilled water, then put in plastic bags with sterilized distilled water at a rate of 1:1 (weight/volume) and kept in the freezer for at least 12 hours at -20 °C.

Table 4. chemical constituents of biogas fertilizer.

Biogas manure	Values	
	First season	Second season
Organic matter %	34	33
Organic carbon %	20	23
Total nitrogen %	1.65	1.64
Total phosphate %	0.85	0.9
Total potassium %	0.35	0.34
Density Kg/m^3	285	260
Moisture %	10	10
Saturation %	210	210

Table 5. Chemical constituents of garlic aqueous extracts fresh cloves.

Constituents		Quantitative Analysis
Potassium		2,127 ppm
Calcium		35.36 ppm
Magnesium		104.65 ppm
Zinc		2.84 ppm
Phosphorous		600.3 ppm
Aluminum		2.315 ppm
Auxins	IAA	210.80 ng ml^{-1} fw
	IBA	304.15 ng ml^{-1} fw
	IPA	627.21 ng ml^{-1} fw
Cytokinins	tZ	54.09 ng ml^{-1} fw
	tZR	76.47 ng ml^{-1} fw
Gibberellins	GA_3	113.72 ng ml^{-1} fw
	GA_4	68.53 ng ml^{-1} fw
	GA_7	173.52 ng ml^{-1} fw

Table 6. Chemical composition of fresh Azolla extracts.

Properties	Quantitative analysis
PH	6.35
EC	1.01
Total nitrogen (ppm)	150
Total Phosphorus (P_2O_5; ppm)	34
Total potassium (K_2O) (ppm)	152
Chemical oxygen demand (COD; mg/l)	12
Biological oxygen demand (BOD; mg/l)	3.1

The plant material was then withdrawn from the refrigerator and allowed to melt at room temperature. The mixture was hardly crushed and blended for 5 minutes, filtered through double layers of cheesecloth, centrifuged at 12,000 rpm for 30 minutes. The resulting solution was kept in the refrigerator at 5 °C until use. The crude extract was considered 100% concentration and serial dilution (50%) was performed using distilled water. (El-Shimi *et al*., 2015).

Yucca extract:

Yucca extract powder was obtained from NPK industries company it was prepared by dissolving 0.3 g of the powder in 1l water as the chemical composition of yucca extract id shown in Table (7).

Harvesting:

The experiments were conducted for six months from April to October of each season. The first cut was conducted on the July 15th and the second cut was conducted on October 15th of both seasons.

Table 7. Chemical composition of yucca extracts.

Constituents	Quantitative analysis mg 100 g^{-1} dw
Potassium	279
Phosphorous	27.8
Magnesium	21.65
Manganese	0.40
Zinc	0.35
Coper	0.10
Vitamin c	21.2
Vitamin B6	0.10
Vitamin K	1.95
Thiamine	0.10
Riboflavin	0.50
Niacin	0.90

Biomass characteristics measure:

Shoots fresh weight biomass kg/ha, shoots dry weight biomass kg/ha and leaves fresh weight biomass kg/ha Leaves dry weight biomass kg/ha.

Chemical constituent attributes:

Photosynthetic pigments determination:

Chlorophyll a, b, total chlorophyll, and carotenoids were determined following (Saric, 1967).

Nutrient element%:

Nitrogen was determined by the modified micro-Kjeldahl method as described by (A.O.A.C., 1980). Phosphorus was determined using the ammonium molybdate method according to (Murphy and Riley, 1962). Potassium % was estimated using flam photometric method according to (Cottenie *et al*., 1982).

Nitrate concentration:

Stevia sampled from the leaves was dried by air to determine nitrate concentration. Nitrate levels in the dry leaves were determined according to the method of (Cataldo *et al*., 1975).

Stevioside content:

The sativoside content in the leaves was estimated by HPLC analysis according to. (Abou-arab *et al*., 2010).

Experimental design:

The experimental layout was a split-plot design arranged with three replicates. Each replicate contained 32 treatments, chemical and three biogas fertilizer levels treatment (T) were assigned in main plots, which were split into eight treatments of natural extracts and five plants were used as an experimental unit. Collected data of both seasons were pooled and the obtained results were analyzed using MSTATC Program.(Bricker, 1991) Means were compared using LSD test at 0.05 level according to (Snedecor and Cochran, 1989).

RESULTS

Shoots fresh and dry weights biomass:

According to data presented in Table (8), all fertilization treatments succeeded in increasing the shoots fresh and dry weights Biomass of *Stevia rebaudiana*, particularly the recommended dose of chemical fertilizer (T_1) in the first cut of both seasons. Although the second cut follows the same pattern as the first cut of both seasons, followed by 50% NPK +5000 kg h^{-1} (T_3) with no major differences. On the other hand, plants treated with the (T2) prescribed dose of biogas fertilizer of both cuts and seasons, produced the lowest fresh and dry weights Biomass.

The results achieved concerning an increment in shoots fresh and dry weights biomass by different natural extracts application methods, garlic aqueous extract as drench addition at 10% (M_4) recorded the highest shoots fresh weight biomass in the first cut of both seasons followed by Azolla aqueous extract at (50%) as drench addition with no significant differences between them in the second cut. Although the shoots dry weights biomass goes in the line with those found in fresh weight biomass as (M_4) have the highest dry weight biomass values of both 1^{st} and 2^{nd} cuts but without significant variance with (M_2) of both seasons. At the same time, the lowest values were recorded by distilled water as drenching or foliar application method (T_7 and T_8) with no significant differences between them.

It is also obvious from Tables that the positive impact of the interaction between the prescribed dose of chemical fertilizer and yucca as a foliar spray ($T_1 \times M_5$) gave the highest plant fresh and dry weights biomass only in the first cut of both seasons. In the second cut registered the highest shoot fresh and dry weights biomass with 50% NPK + 5000 kg h^{-1} biogas, combined with the addition of the drenched garlic aqueous extract ($T_3 \times M_4$) of both seasons. In the contrast, the lightest fresh and dry weights biomass was recorded by the interaction of recommended dose of biogas fertilizer and distilled water as a foliar spray ($T_2 \times M_7$) in the first cut of both seasons while the second cut was in the same line with the first but with distilled water as drench addition ($T_2 \times M_8$).

Leaves fresh and dry weights biomass:

Data presented in Table (9) states that using recommended dose of chemical fertilizer (T_1) recorded the heaviest leaves fresh and dry wights biomass only in the first cut of both seasons. Although the second cut recorded the highest leaves fresh weights of both seasons by 50% NPK +5000 kg h-1 biogas fertilizer (T_3). However, in the case of the leaves dry weight biomass there were non-significant differences with (T_1) in the first season only. Whereas the lightest leaves fresh and dry weights biomass was observed during both seasons by recommended dose of biogas fertilizer (T_2).

It is quite evident as shown from tabulated data that the response of leaves fresh and dry weights biomass to natural extracts were enhanced by Azolla aqueous extract as drench addition (M_2) which registered the highest values of both cuts and seasons. However, distilled water as foliar or drench addition (M_7 or M_8) gave the lowest values in this regard.

In both seasons, the response of stevia leaves fresh and dry weights biomass to the interaction of fertilization and natural extract

Table 8. Effect of biogas, chemical fertilizer, and natural extracts and their interaction on shoots fresh and dry wights biomass of *Stevia rebaudiana* L. plants in 2019 and 2020 seasons for two cuts.

Fertilization	Natural ex. Methods app	Shoots fresh weight biomass kg/ha				Shoots dry weight biomass kg/ha			
		2019		2020		2019		2020	
		1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut
T1		9870.0	10502.5	9401.9	10112.9	2598.7	2786.0	2446.0	2655.1
T2		3951.8	4788.0	3511.3	4101.7	873.7	1117.4	806.7	950.9
T3		9325.5	10547.6	8970.1	10074.7	2203.0	2805.8	2180.3	2620.5
T4		8582.9	10068.0	8067.4	8821.9	2087.8	2351.1	2033.2	2223.1
LSD at 0.05		178.21	103.45	163.38	175.14	39.16	27.71	50.89	53.87
	M1	8256.6	8862.5	7938.1	8339.4	1921.5	2253.4	1973.2	2117.4
	M2	8961.1	9665.7	8524.0	9115.7	2325.5	2553.7	2272.7	2456.6
	M3	8352.0	9286.8	8067.3	8749.5	2044.9	2385.2	2055.5	2247.0
	M4	9182.6	9707.7	8772.5	9233.9	2369.9	2594.1	2287.9	2482.8
	M5	8131.9	8960.1	8100.0	8251.4	2024.8	2317.8	1919.5	2108.9
	M6	8078.7	8785.4	7951.6	7845.7	1855.9	2244.5	1919.2	2000.9
	M7	6273.2	8295.3	5297.9	7346.9	1481.0	1885.9	1264.8	1732.9
	M8	6224.4	8248.8	5250.1	7339.9	1503.0	1885.9	1239.8	1752.5
LSD at 0.05		170.14	138.52	179.95	163.16	53.27	41.94	45.23	40.05
T1	M1	10133.3	10269.2	9734.5	10017.6	2584.0	2670.0	2501.8	2597.3
	M2	10533.8	10769.2	9966.7	10210.6	2997.6	2940.0	2784.5	2777.3
	M3	10264.3	10365.7	10434.7	10007.2	2760.0	2778.0	2728.1	2647.2
	M4	10690.5	10821.8	10507.5	10352.7	2918.5	2976.0	2691.0	2847.0
	M5	10836.0	11036.6	10666.8	10725.6	3034.1	2982.0	2859.1	2907.6
	M6	10221.6	10860.0	9890.9	10004.4	2555.4	2886.0	2429.3	2714.0
	M7	8214.9	9973.5	7138.1	9846.5	1988.0	2502.0	1827.4	2376.5
	M8	8065.5	9923.8	6876.4	9738.5	1951.9	2553.6	1746.6	2374.1
T2	M1	3599.9	4410.4	3581.2	3647.3	828.0	1054.1	838.0	850.2
	M2	4600.0	5582.2	4099.6	4823.4	1104.0	1378.8	988.0	1245.6
	M3	4222.2	5292.5	3493.7	4740.9	950.0	1270.2	828.0	1116.9
	M4	5373.9	5605.4	4685.7	4893.4	1236.0	1440.6	1148.0	1257.6
	M5	3723.8	4718.2	3157.9	4171.6	782.0	1038.0	720.0	913.8
	M6	3384.5	4609.8	3169.6	3968.2	748.0	1020.0	710.0	873.0
	M7	3270.0	4085.1	2883.5	3391.1	654.0	884.4	594.0	700.8
	M8	3440.0	4000.6	3019.2	3177.8	688.0	852.7	628.0	649.2
T3	M1	9596.9	10155.6	9618.0	10114.7	1976.0	2742.0	2313.1	2731.0
	M2	10455.6	11168.5	10359.1	10774.8	2739.4	3090.0	2732.2	2935.4
	M3	9713.3	11027.0	9824.4	10690.0	2186.0	2856.0	2468.6	2818.6
	M4	10577.2	11280.0	10214.1	10969.1	2803.0	3112.3	2717.4	2953.9
	M5	9027.7	10500.0	10410.5	9954.2	2137.5	2874.0	2106.6	2592.0
	M6	8857.4	10232.6	9429.4	9804.7	1736.0	2712.0	2282.9	2520.0
	M7	8184.0	10024.1	5971.8	9113.9	1998.0	2552.2	1421.3	2159.3
	M8	8192.0	9993.1	5933.9	9176.3	2048.0	2508.2	1400.4	2253.5
T4	M1	9696.2	10615.0	8818.6	9578.0	2298.0	2547.6	2239.9	2291.3
	M2	10255.0	11142.9	9670.6	10654.1	2461.2	2805.8	2586.2	2868.2
	M3	9208.1	10461.9	8516.3	9559.9	2283.6	2636.4	2197.2	2405.3
	M4	10088.6	11123.4	9682.8	10720.3	2522.2	2847.6	2595.0	2872.8
	M5	8940.0	9585.5	8164.9	8154.2	2145.6	2377.2	1992.2	2022.2
	M6	9851.2	9439.2	9316.4	7605.6	2384.0	2359.8	2254.6	1896.7
	M7	5424.0	9098.6	5198.3	7036.0	1284.0	1605.0	1216.4	1695.1
	M8	5200.0	9077.5	5171.0	7267.1	1324.0	1629.0	1184.2	1733.4
LSD at 0.05		340.28	277.03	359.91	326.32	106.54	83.88	90.45	80.10

*** Means in the same column for each trait are the average of two independent experiments (n = 3). Values significantly differ from each other according to LSD test at P=0.05.**

*** (T1) 100 % Recommended dose of NPK (100:50:50 Kg/ha), (T2) 100% Recommended dose of Biogas Slurry (BGS) (6000 kg/ha), (T3) 50% NPK+ BGS (5000 kg/ha), (T4) 50 % NPK + BGS (3000 kg /ha), (M1) Azolla aqueous extract (50%) as a foliar spray, (M2) Azolla aqueous extract (50%) as drench addition, (M3) garlic aqueous extract (10%) as a foliar spray, (M4) garlic aqueous extract (10%) as drench addition, (M5) yucca aqueous extract (0.3 g/l) as a foliar spray, (M6) yucca aqueous extract (0.3 g/l) as drench addition, (M7) distilled water as a foliar spray, (M8) distilled water as drench addition.**

Table 9. Effect of biogas, chemical fertilizer, and natural extracts and their interaction on leaves fresh and dry weights biomass of *Stevia rebaudiana* L. plants in 2019 and 2020 seasons for two cuts.

Fertilization	Natural ex. Methods app	Leaves fresh weights biomass kg/ha				Leaves dry weights biomass kg/ha			
		2019		2020		2019		2020	
		1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut
T1		6362.8	6450.6	6064.4	6223.4	1722.3	1745.2	1641.5	1682.8
T2		1982.1	2613.6	1790.9	2114.7	433.1	570.4	391.8	462.1
T3		5995.4	6540.4	5435.3	6411.6	1590.4	1732.9	1458.3	1708.2
T4		5698.7	5924.7	5223.28	5902.71	1352.9	1408.7	1310.8	1475.1
LSD at 0.05		84.02	55.99	76.6	45.8	20.22	13.06	20.56	12.19
	M1	5169.4	5732.2	4878.7	5399.9	1362.1	1502.6	1316.3	1455.6
	M2	5890.7	6305.9	5624.3	5887.1	1617.2	1717.1	1578.0	1648.2
	M3	5087.5	5699.3	4651.6	5355.6	1324.0	1471.4	1253.7	1438.8
	M4	5728.6	6045.4	5520.8	5710.6	1526.2	1610.4	1529.4	1574.4
	M5	4881.8	5288.1	4486.1	5024.0	1203.3	1297.9	1103.8	1235.9
	M6	4808.0	5078.8	4209.5	4884.3	1174.9	1235.4	1029.2	1190.0
	M7	4287.1	4448.9	3830.8	4529.9	999.0	1034.5	894.8	1054.1
	M8	4224.9	4460.0	3825.9	4513.6	990.7	1045.0	899.7	1059.4
LSD at 0.05		82.47	68.70	78.8	65.1	20.52	17.14	20.02	16.82
T1	M1	6374.4	6580.8	6321.6	6425.3	1784.8	1842.6	1770.0	1799.1
	M2	7224.2	7358.4	6864.0	6939.6	2095.0	2133.9	1990.6	2012.5
	M3	6681.6	6716.4	6142.80	6320.64	1870.8	1880.6	1720.0	1769.8
	M4	7004.4	6989.5	6770.4	6706.6	1996.3	1992.0	1929.6	1911.4
	M5	6310.6	6224.6	5947.7	6119.5	1640.7	1618.4	1546.4	1591.1
	M6	6363.4	5995.2	5852.9	5957.3	1654.5	1558.8	1521.7	1548.9
	M7	5660.6	5856.0	5354.2	5622.0	1415.2	1464.0	1338.5	1405.5
	M8	5283.1	5883.8	5261.3	5696.6	1320.8	1471.0	1315.3	1424.2
T2	M1	2354.2	3045.1	2068.8	2328.5	494.4	639.5	434.4	489.0
	M2	2572.8	3451.7	2491.9	2845.4	591.7	793.9	573.1	654.5
	M3	2107.2	2875.7	1952.4	2507.8	463.6	632.6	429.5	551.7
	M4	2793.6	3169.4	2524.8	2643.84	642.5	729.0	580.7	608.1
	M5	1704.0	2460.0	1574.4	1948.3	374.9	541.2	346.4	428.6
	M6	1622.4	2275.2	1358.4	1807.2	356.9	500.5	298.8	397.6
	M7	1339.2	1834.6	1137.6	1451.5	267.8	366.9	227.5	290.3
	M8	1363.2	1797.1	1219.2	1385.3	272.6	359.4	243.8	277.1
T3	M1	6069.6	6753.6	5722.1	6609.6	1699.5	1891.0	1602.2	1850.7
	M2	7035.3	7579.2	6718.3	7046.9	2065.6	2198.0	1981.9	2078.8
	M3	5950.8	6854.4	5427.8	6683.0	1586.9	1816.4	1492.7	1837.8
	M4	6458.4	7387.2	6577.2	6950.6	1808.4	2068.4	1874.5	1953.1
	M5	5984.2	6578.2	5012.6	6296.4	1526.0	1677.4	1278.2	1605.6
	M6	5888.9	6439.0	4824.5	6140.2	1472.2	1609.7	1206.1	1535.0
	M7	5238.2	5379.1	4578.5	5826.2	1257.2	1291.0	1098.8	1398.3
	M8	5338.1	5352.2	4621.2	5739.6	1307.8	1311.3	1132.2	1406.2
T4	M1	5879.5	6549.1	5402.4	6236.2	1469.9	1637.3	1458.7	1683.8
	M2	6730.6	6834.2	6423.1	6716.4	1716.3	1742.7	1766.4	1847.0
	M3	5610.2	6350.9	5083.2	5911.0	1374.5	1556.0	1372.5	1596.0
	M4	6657.8	6635.5	6210.7	6541.2	1657.8	1652.2	1732.8	1825.0
	M5	5528.4	5889.6	5409.6	5731.7	1271.5	1354.6	1244.2	1318.3
	M6	5357.3	5605.9	4802.4	5632.6	1216.1	1272.5	1090.1	1278.6
	M7	4910.4	4725.8	4252.8	5220.0	1055.7	1016.1	914.4	1122.3
	M8	4915.2	4806.7	4201.9	5232.7	1061.7	1038.3	907.6	1130.3
LSD at 0.05		164.94	137.40	157.6	130.3	41.04	34.27	40.03	33.65

*** Means in the same column for each trait are the average of two independent experiments (n = 3). Values significantly differ from each other according to LSD test at P=0.05.**

*** (T1) 100 % Recommended dose of NPK (100:50:50 Kg/ha), (T2) 100% Recommended dose of Biogas Slurry (BGS) (6000 kg/ha), (T3) 50% NPK+ BGS (5000 kg/ha), (T4) 50 % NPK + BGS (3000 kg /ha), (M1) Azolla aqueous extract (50%) as a foliar spray, (M2) Azolla aqueous extract (50%) as drench addition, (M3) garlic aqueous extract (10%) as a foliar spray, (M4) garlic aqueous extract (10%) as drench addition, (M5) yucca aqueous extract (0.3 g/l) as a foliar spray, (M6) yucca aqueous extract (0.3 g/l) as drench addition, (M7) distilled water as a foliar spray, (M8) distilled water as drench addition.**

application methods followed the same pattern previously detected as the recommended dose of chemical fertilizer and Azolla aqueous extract (50%) as drench addition ($T_1 \times M_2$) in the first cut only. The second cut also had the highest levels, in combination with a 50 % NPK +5000 kg h^{-1}, with Azolla aqueous extract as drench addition ($T_3 \times M_2$) of both seasons. On the other hand, the interaction of the recommended dose of biogas fertilizer and distilled water as a foliar spray ($T_2 \times M_7$) in the first cut of both seasons resulted in the lowest fresh and dry weights of leaves Biomass, while the second cut followed the same trend as the first cut but with distilled water as a drench addition ($T_2 \times M_8$).

Leaf nitrogen percentage (%):

Tabulated data in Table (10) revealed that all investigated treatments especially recommended dose of chemical fertilizer (T_1) recorded the highest nitrogen percentage of both cuts in 1st season as well as 2nd season. Although 50% NPK +5000 kg h^{-1} biogas fertilizer (T_3) registered the highest nitrogen percentage in the second cut on the second season which had no significant differences with (T_1). In contrast, the lowest values of parameters mentioned above were registered by 100 % recommended dose of biogas fertilizer (T_2) of both cuts and seasons.

Regarding natural extracts treatments, data state that plants treated with Azolla aqueous extract as drench addition at 50% (M_2) gave the highest nitrogen percentage of the two cuts as well as both seasons without significant values with (M_4) in the first cut only of both seasons while the lowest values were recorded by Azolla aqueous extract (M_2) of both cuts and seasons.

Additionally, the interaction between recommended dose of chemical fertilizer with garlic aqueous extract ($T_1 \times M_4$) recorded the highest nitrogen percentage in the first cut only while the second cut of both seasons registered by 50% NPK+5000 kg h^{-1} combining with Azolla aqueous extract ($T_3 \times M_2$). On the reverse, the lowest nitrogen content values were recorded by the interaction between a recommended dose of biogas fertilizer and distilled water as a foliar spray as well as drench addition ($T_2 \times M_7$) without significant differences between them.

Leaf nitrate content:

According to data presented in Table (10), the recommended dose of chemical fertilizer (T_1) recorded the highest nitrate content in stevia leaf. In contrast, the recommended dose of biogas fertilizer (T_2) registered the lowest nitrate values in both cuts in both seasons.

Regarding natural extract treatments and their effect on nitrate content, results showed that plants treated with Azolla aqueous extract as drench addition (M_2) of both cuts and seasons recorded the lowest nitrate content compared to distilled water as a foliar spray (M_7) as well as distilled water as drench addition (M_8) without significant differences between them.

Moreover, the effect of the interaction between fertilization and natural extracts showed that the recommended dose of biogas fertilizer with Azolla extract as drench addition ($T_2 \times M_2$) registered the lowest nitrate values compared to $T_1 \times M_7$ treatment which recorded the highest nitrate content in both cuts in both seasons.

Leaf phosphorus and potassium percentage (%):

Concerning the response of the phosphorus and potassium to the different investigated fertilization treatments, data presented in Table (11) show that recommended dose of the chemical fertilizer (T_1) recorded the highest phosphorus and potassium leaf percentage in the first cut only of both seasons while the second cut of both seasons registered the highest values by using treatment 50% NPK + 5000 kg h^{-1} (T_3) biogas fertilizer. On the contrary, the lowest values were recorded by recommended dose of biogas fertilizer (T_2) in the first cut as well as the second cut of both seasons.

Table 10. Effect of biogas, chemical fertilizer, and natural extracts and their interaction on nitrogen (%) and nitrate content of *Stevia rebaudiana* L. plants in 2019 and 2020 seasons for two cuts.

Fertilization	Natural ex. Methods app	N (%)				Nitrate mg/kg dw			
		2019		2020		2019		2020	
		1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut
T1		1.46	1.55	1.41	1.50	25.88	28.04	27.88	28.79
T2		0.82	0.89	0.71	0.76	16.00	15.46	16.21	17.46
T3		1.39	1.52	1.37	1.51	21.13	25.00	22.17	27.88
T4		1.27	1.38	1.27	1.39	23.29	26.00	22.79	27.50
LSD at 0.05		0.02	0.01	0.01	0.01	0.41	0.71	0.40	0.51
	M1	1.29	1.45	1.22	1.40	20.83	23.25	21.00	25.08
	M2	1.42	1.52	1.35	1.50	19.50	22.50	21.00	24.17
	M3	1.33	1.39	1.27	1.34	21.25	23.50	22.17	25.33
	M4	1.40	1.46	1.36	1.46	21.75	23.50	21.75	25.17
	M5	1.22	1.34	1.14	1.26	22.58	24.25	22.42	26.08
	M6	1.18	1.28	1.10	1.23	21.00	22.58	22.75	24.33
	M7	1.04	1.14	1.04	1.08	23.00	24.83	23.58	26.50
	M8	1.02	1.13	1.04	1.06	22.67	24.58	23.42	26.58
LSD at 0.05		0.02	0.02	0.01	0.01	0.57	0.66	0.58	0.61
T1	M1	1.47	1.65	1.44	1.62	25.33	27.33	25.67	28.33
	M2	1.66	1.73	1.54	1.65	24.00	26.00	26.00	26.67
	M3	1.54	1.63	1.49	1.55	25.33	27.00	27.67	28.33
	M4	1.71	1.65	1.63	1.68	25.67	27.67	27.33	28.33
	M5	1.42	1.58	1.37	1.47	26.33	28.67	27.00	28.67
	M6	1.37	1.47	1.33	1.43	25.67	26.67	28.67	28.00
	M7	1.29	1.40	1.25	1.31	27.33	30.67	30.33	31.00
	M8	1.26	1.32	1.25	1.31	27.33	30.33	30.33	31.00
T2	M1	0.92	1.06	0.76	0.81	16.67	16.00	14.67	16.33
	M2	0.96	1.10	0.85	0.96	14.33	14.67	15.67	16.00
	M3	0.95	0.91	0.82	0.79	16.00	15.67	16.33	16.67
	M4	0.98	0.95	0.92	0.90	16.33	16.33	16.00	17.67
	M5	0.83	0.93	0.63	0.74	17.33	16.00	15.67	18.67
	M6	0.76	0.84	0.57	0.72	15.00	14.67	18.00	16.33
	M7	0.58	0.65	0.57	0.58	16.67	15.33	16.67	18.67
	M8	0.57	0.68	0.56	0.57	15.67	15.00	16.67	19.33
T3	M1	1.45	1.64	1.41	1.66	18.67	24.33	21.33	27.67
	M2	1.68	1.78	1.59	1.74	18.33	24.67	20.33	27.00
	M3	1.51	1.57	1.49	1.57	20.33	25.67	22.00	28.67
	M4	1.56	1.75	1.53	1.71	21.33	24.67	21.67	28.00
	M5	1.36	1.46	1.32	1.45	22.67	25.67	23.33	28.67
	M6	1.32	1.42	1.28	1.40	21.00	24.33	21.67	27.00
	M7	1.15	1.27	1.16	1.26	23.33	25.33	23.67	28.00
	M8	1.14	1.27	1.16	1.26	23.33	25.33	23.33	28.00
T4	M1	1.30	1.45	1.27	1.50	22.67	25.33	22.33	28.00
	M2	1.37	1.47	1.40	1.63	21.33	24.67	22.00	27.00
	M3	1.33	1.45	1.30	1.47	23.33	25.67	22.67	27.67
	M4	1.34	1.48	1.36	1.57	23.67	25.33	22.00	26.67
	M5	1.27	1.37	1.23	1.37	24.00	26.67	23.67	28.33
	M6	1.26	1.37	1.22	1.36	22.33	24.67	22.67	26.00
	M7	1.15	1.23	1.19	1.15	24.67	28.00	23.67	28.33
	M8	1.13	1.25	1.18	1.11	24.33	27.67	23.33	28.00
LSD at 0.05		0.03	0.03	0.03	0.02	1.14	1.33	1.16	1.23

*** Means in the same column for each trait are the average of two independent experiments (n = 3). Values significantly differ from each other according to LSD test at P=0.05.**

*** (T1) 100 % Recommended dose of NPK (100:50:50 Kg/ha), (T2) 100% Recommended dose of Biogas Slurry (BGS) (6000 kg/ha), (T3) 50% NPK+ BGS (5000 kg/ha), (T4) 50 % NPK + BGS (3000 kg /ha), (M1) Azolla aqueous extract (50%) as a foliar spray, (M2) Azolla aqueous extract (50%) as drench addition, (M3) garlic aqueous extract (10%) as a foliar spray, (M4) garlic aqueous extract (10%) as drench addition, (M5) yucca aqueous extract (0.3 g/l) as a foliar spray, (M6) yucca aqueous extract (0.3 g/l) as drench addition, (M7) distilled water as a foliar spray, (M8) distilled water as drench addition.**

Table 11. Effect of biogas, chemical fertilizer, and natural extracts and their interaction on phosphorus and potassium content of *Stevia rebaudiana* L. plants in 2019 and 2020 seasons for two cuts.

Fertilization	Natural ex. Methods app	P (%)				K (%)			
		2019		2020		2019		2020	
		1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut
T1		0.212	0.220	0.204	0.216	2.329	2.377	2.386	2.407
T2		0.154	0.157	0.140	0.149	1.168	1.245	1.295	1.335
T3		0.205	0.229	0.198	0.223	2.286	2.387	2.324	2.418
T4		0.171	0.211	0.180	0.213	1.745	2.238	1.757	2.276
LSD at 0.05		0.006	0.003	0.003	0.003	0.012	0.010	0.006	0.008
	M1	0.184	0.194	0.162	0.194	1.897	2.124	1.988	2.164
	M2	0.202	0.221	0.198	0.220	2.073	2.182	2.056	2.228
	M3	0.191	0.204	0.180	0.205	1.931	2.133	2.016	2.159
	M4	0.211	0.227	0.204	0.222	2.099	2.221	2.118	2.254
	M5	0.189	0.212	0.189	0.211	1.846	2.052	1.899	2.107
	M6	0.178	0.204	0.180	0.205	1.821	2.008	1.861	2.078
	M7	0.167	0.184	0.166	0.174	1.697	1.885	1.793	1.936
	M8	0.165	0.186	0.165	0.172	1.693	1.888	1.795	1.947
LSD at 0.05		0.004	0.004	0.005	0.003	0.013	0.010	0.011	0.011
T1	M1	0.215	0.205	0.193	0.210	2.343	2.413	2.407	2.450
	M2	0.225	0.244	0.220	0.236	2.487	2.453	2.483	2.487
	M3	0.213	0.216	0.197	0.220	2.360	2.427	2.437	2.433
	M4	0.235	0.238	0.227	0.244	2.510	2.480	2.517	2.527
	M5	0.218	0.236	0.215	0.227	2.290	2.367	2.370	2.393
	M6	0.206	0.226	0.210	0.225	2.277	2.333	2.337	2.347
	M7	0.194	0.196	0.188	0.186	2.197	2.270	2.267	2.307
	M8	0.192	0.195	0.185	0.183	2.170	2.273	2.273	2.310
T2	M1	0.150	0.161	0.139	0.146	1.217	1.260	1.320	1.357
	M2	0.183	0.164	0.154	0.165	1.240	1.320	1.380	1.410
	M3	0.163	0.169	0.141	0.155	1.223	1.297	1.337	1.380
	M4	0.173	0.180	0.156	0.164	1.260	1.370	1.420	1.437
	M5	0.151	0.155	0.145	0.148	1.190	1.240	1.270	1.323
	M6	0.145	0.145	0.130	0.143	1.160	1.220	1.243	1.290
	M7	0.134	0.137	0.127	0.137	1.023	1.127	1.190	1.227
	M8	0.135	0.144	0.126	0.134	1.030	1.128	1.197	1.260
T3	M1	0.203	0.216	0.163	0.215	2.290	2.443	2.353	2.443
	M2	0.207	0.252	0.215	0.244	2.463	2.543	2.417	2.563
	M3	0.203	0.224	0.193	0.228	2.323	2.423	2.383	2.407
	M4	0.233	0.257	0.222	0.247	2.477	2.563	2.473	2.573
	M5	0.214	0.231	0.207	0.237	2.247	2.370	2.317	2.380
	M6	0.204	0.223	0.203	0.229	2.220	2.340	2.260	2.360
	M7	0.191	0.215	0.193	0.193	2.133	2.203	2.197	2.310
	M8	0.188	0.213	0.185	0.192	2.137	2.207	2.195	2.310
T4	M1	0.167	0.195	0.153	0.204	1.737	2.380	1.870	2.407
	M2	0.193	0.225	0.203	0.235	2.100	2.410	1.943	2.450
	M3	0.183	0.207	0.191	0.217	1.817	2.387	1.907	2.417
	M4	0.203	0.233	0.211	0.234	2.150	2.470	2.060	2.480
	M5	0.173	0.226	0.187	0.234	1.657	2.230	1.640	2.330
	M6	0.157	0.220	0.177	0.224	1.627	2.140	1.603	2.317
	M7	0.147	0.190	0.157	0.179	1.433	1.940	1.517	1.900
	M8	0.143	0.194	0.163	0.178	1.437	1.943	1.513	1.907
LSD at 0.05		0.008	0.007	0.009	0.006	0.026	0.020	0.022	0.023

*** Means in the same column for each trait are the average of two independent experiments (n = 3). Values significantly differ from each other according to LSD test at P=0.05.**

*** (T1) 100 % Recommended dose of NPK (100:50:50 Kg/ha), (T2) 100% Recommended dose of Biogas Slurry (BGS) (6000 kg/ha), (T3) 50% NPK+ BGS (5000 kg/ha), (T4) 50 % NPK + BGS (3000 kg /ha), (M1) Azolla aqueous extract (50%) as a foliar spray, (M2) Azolla aqueous extract (50%) as drench addition, (M3) garlic aqueous extract (10%) as a foliar spray, (M4) garlic aqueous extract (10%) as drench addition, (M5) yucca aqueous extract (0.3 g/l) as a foliar spray, (M6) yucca aqueous extract (0.3 g/l) as drench addition, (M7) distilled water as a foliar spray, (M8) distilled water as drench addition.**

Referring to the response of phosphorus and potassium to natural extracts data presented in Table (11) display obviously that garlic extract (M_4) recorded the highest values of both cuts in the first and second cuts meanwhile the lowest values were recorded by distilled water as foliar spray or drench addition (T_7 or T_8) without significant difference between them.

As for the interaction between the two factors, the results presented in Table (11) revealed that the interaction between recommended dose of chemical fertilizer and garlic aqueous extract as drench addition ($T_1 \times M_4$) registered the highest phosphorus and potassium leave percentage in the first cut only of both seasons. On the other hand, the interaction between 50% NPK + 5000 kg h^{-1} biogas fertilizer and garlic aqueous extract as drench addition ($T_3 \times M_4$) recorded the highest values in the second cut of both seasons. Meanwhile, the lowest values were recorded by the interaction between a recommended dose of biogas fertilizer and distilled water as a foliar spray ($T_2 \times M_7$) as well as drench addition ($T_2 \times M_8$) without significant differences between them.

Chlorophyll a and chlorophyll b content:

Data presented in Table (12) display obviously that investigated treatments with any biogas fertilizer and natural extracts and their interaction significantly increased the chlorophyll content. As for 50% NPK+5000 kg h^{-1} biogas fertilizer (T_3) recorded the highest chlorophyll a and b content in the first and second cuts of both seasons. In contrast, the lowest chlorophyll a and b content values were recorded by recommended dose of biogas fertilizer (T_2).

Concerning natural extracts the highest content of chlorophyll a and b contents were recorded by Azolla aqueous extract as drench addition (M_2) of both cuts in the first and second seasons while the lowest chlorophyll a and b contents were registered by distilled water (M_7) as well as foliar or drench addition (M_8) without significant variation between them.

On the other side, the interaction between the recommended dose of chemical fertilizer and Azolla aqueous extract as drench addition ($T_1 \times M_2$) registered the highest chlorophyll a and b in the first cut of both seasons while in the second cut of both seasons the highest values were recorded by the combination between 50% NPK +5000 kg h^{-1} with Azolla aqueous extract as drench addition ($T_3 \times M_2$). However, the minimum values in chlorophyll a and b registered coming from the interaction between a recommended dose of biogas fertilizer (T_2) with distilled water as foliar as well as drench addition without significant variation between them.

Carotenoids and total chlorophylls content:

Data presented Table (13) show obviously the response of carotenoids and total chlorophylls to different fertilization rates of recommended dose of chemical fertilizer (T_1) in the first cut only in the first and second seasons recorded the highest values of the carotenoids content while the second cut of both seasons registered the highest values by 50% NPK +5000 kg h^{-1} biogas fertilizer (T_3), likewise total chlorophyll which took the same trend with the second cut of carotenoids content of both cuts and seasons. On the contrary, the lowest values of carotenoids, as well as total chlorophylls, were recorded by the recommended dose of biogas fertilizer of both cuts and seasons.

Regarding natural extracts application methods, the highest values of both carotenoids and total chlorophylls were recorded by Azolla aqueous extract as drench addition while the lowest values were recorded by distilled water as foliar or drench addition without significant differences between them.

On the other hand, the interaction between of recommended dose of chemical fertilizer with Azolla aqueous extract as drench addition ($T_1 \times M_2$) in the first cut only recorded the highest carotenoids and total

Table 12. Effect of biogas, chemical fertilizer, and natural extracts and their interaction on chlorophyll a and chlorophyll b of *Stevia rebaudiana* L. plants in 2019 and 2020 seasons for two cuts.

Fertilization	Natural ex. Methods app	Chl a (mg g^{-1} fw)				Chl b (mg g^{-1} fw)			
		2019		2020		2019		2020	
		1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut
T1		0.3572	0.3552	0.3656	0.3628	0.1141	0.1184	0.1179	0.1209
T2		0.3121	0.3214	0.3228	0.3284	0.1038	0.1071	0.1077	0.1094
T3		0.3555	0.3744	0.3673	0.3823	0.1186	0.1238	0.1227	0.1276
T4		0.3330	0.3415	0.3397	0.3525	0.1118	0.1138	0.1157	0.1175
LSD at 0.05		0.0045	0.0017	0.0027	0.0029	0.0007	0.0007	0.0005	0.0009
	M1	0.3464	0.3598	0.3548	0.3678	0.1155	0.1199	0.1193	0.1226
	M2	0.4102	0.4255	0.4204	0.4337	0.1367	0.1418	0.1401	0.1446
	M3	0.3559	0.3669	0.3675	0.3771	0.1186	0.1223	0.1221	0.1257
	M4	0.3985	0.4162	0.4134	0.4246	0.1342	0.1376	0.1384	0.1415
	M5	0.3684	0.3428	0.3679	0.3524	0.1148	0.1143	0.1197	0.1175
	M6	0.3408	0.3314	0.3493	0.3563	0.1127	0.1105	0.1164	0.1188
	M7	0.2473	0.2705	0.2593	0.2692	0.0826	0.0902	0.0860	0.0898
	M8	0.2483	0.2718	0.2580	0.2709	0.0817	0.0897	0.0860	0.0906
LSD at 0.05		0.0044	0.0030	0.0036	0.0024	0.0010	0.0011	0.0007	0.0008
T1	**M1**	0.3677	0.3667	0.3753	0.3713	0.1226	0.1222	0.1251	0.1238
	M2	0.4290	0.4410	0.4390	0.4483	0.1430	0.1470	0.1463	0.1494
	M3	0.3847	0.3813	0.3957	0.4029	0.1282	0.1271	0.1319	0.1343
	M4	0.4250	0.4290	0.4333	0.4433	0.1417	0.1430	0.1444	0.1478
	M5	0.4070	0.3347	0.4210	0.3447	0.1038	0.1116	0.1084	0.1149
	M6	0.3273	0.3250	0.3313	0.3420	0.1056	0.1083	0.1104	0.1140
	M7	0.2580	0.2817	0.2643	0.2727	0.0860	0.0939	0.0881	0.0909
	M8	0.2590	0.2820	0.2650	0.2773	0.0821	0.0940	0.0883	0.0924
T2	**M1**	0.3183	0.3343	0.3167	0.3400	0.1061	0.1114	0.1097	0.1133
	M2	0.3830	0.3920	0.3930	0.4017	0.1277	0.1307	0.1310	0.1339
	M3	0.3280	0.3390	0.3417	0.3370	0.1093	0.1130	0.1121	0.1123
	M4	0.3720	0.3797	0.3850	0.3843	0.1240	0.1266	0.1283	0.1281
	M5	0.3180	0.3247	0.3343	0.3300	0.1060	0.1082	0.1114	0.1100
	M6	0.3070	0.3133	0.3187	0.3250	0.1023	0.1044	0.1062	0.1083
	M7	0.2354	0.2430	0.2487	0.2539	0.0785	0.0810	0.0811	0.0843
	M8	0.2353	0.2450	0.2443	0.2550	0.0769	0.0817	0.0814	0.0850
T3	**M1**	0.3590	0.3870	0.3763	0.4017	0.1197	0.1290	0.1254	0.1339
	M2	0.4257	0.4573	0.4357	0.4610	0.1419	0.1524	0.1452	0.1537
	M3	0.3640	0.3940	0.3757	0.4043	0.1213	0.1313	0.1252	0.1348
	M4	0.4183	0.4533	0.4250	0.4507	0.1394	0.1467	0.1439	0.1502
	M5	0.3873	0.3487	0.3997	0.3587	0.1291	0.1162	0.1332	0.1196
	M6	0.3833	0.3357	0.3933	0.3953	0.1278	0.1119	0.1311	0.1318
	M7	0.2530	0.3090	0.2683	0.2930	0.0850	0.1030	0.0894	0.0977
	M8	0.2537	0.3103	0.2640	0.2940	0.0846	0.0997	0.0880	0.0989
T4	**M1**	0.3407	0.3510	0.3510	0.3583	0.1136	0.1170	0.1170	0.1194
	M2	0.4030	0.4117	0.4140	0.4237	0.1343	0.1372	0.1380	0.1412
	M3	0.3470	0.3533	0.3570	0.3640	0.1157	0.1178	0.1190	0.1213
	M4	0.3787	0.4027	0.4104	0.4200	0.1318	0.1342	0.1368	0.1400
	M5	0.3613	0.3630	0.3167	0.3763	0.1204	0.1210	0.1256	0.1254
	M6	0.3453	0.3517	0.3540	0.3630	0.1151	0.1172	0.1180	0.1210
	M7	0.2430	0.2483	0.2557	0.2573	0.0810	0.0828	0.0852	0.0857
	M8	0.2450	0.2500	0.2587	0.2573	0.0824	0.0833	0.0862	0.0861
LSD at 0.05		0.0088	0.0059	0.0071	0.0048	0.0019	0.0023	0.0015	0.0016

*** Means in the same column for each trait are the average of two independent experiments (n = 3). Values significantly differ from each other according to LSD test at P=0.05.**

*** (T1) 100 % Recommended dose of NPK (100:50:50 Kg/ha), (T2) 100% Recommended dose of Biogas Slurry (BGS) (6000 kg/ha), (T3) 50% NPK+ BGS (5000 kg/ha), (T4) 50 % NPK + BGS (3000 kg /ha), (M1) Azolla aqueous extract (50%) as a foliar spray, (M2) Azolla aqueous extract (50%) as drench addition, (M3) garlic aqueous extract (10%) as a foliar spray, (M4) garlic aqueous extract (10%) as drench addition, (M5) yucca aqueous extract (0.3 g/l) as a foliar spray, (M6) yucca aqueous extract (0.3 g/l) as drench addition, (M7) distilled water as a foliar spray, (M8) distilled water as drench addition.**

Table 13. Effect of biogas, chemical fertilizer, and natural extracts and their interaction on carotenoids and Total chlorophylls of *Stevia rebaudiana* L. plants in 2019 and 2020 seasons for two cuts.

Fertilization	Natural ex. Methods app	Carotenoids (mg g^{-1} fw)				Total chlorophylls (mg g^{-1} fw)			
		2019		2020		2019		2020	
		1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut
T1		0.1300	0.0637	0.0812	0.0843	0.4713	0.4736	0.4835	0.4838
T2		0.0895	0.0525	0.0414	0.0427	0.4160	0.4285	0.4305	0.4378
T3		0.1039	0.0691	0.0560	0.0905	0.4741	0.4982	0.4899	0.5099
T4		0.0971	0.0592	0.0491	0.0508	0.4448	0.4553	0.4554	0.4700
LSD at 0.05		0.0009	0.0007	0.0005	0.0010	0.0049	0.0021	0.0027	0.0038
	M1	0.1083	0.0653	0.0601	0.0709	0.4619	0.4797	0.4741	0.4904
	M2	0.1296	0.0872	0.0810	0.0922	0.5469	0.5673	0.5606	0.5782
	M3	0.1115	0.0676	0.0629	0.0740	0.4746	0.4892	0.4896	0.5027
	M4	0.1271	0.0829	0.0792	0.0899	0.5327	0.5538	0.5518	0.5661
	M5	0.1077	0.0596	0.0605	0.0658	0.4833	0.4570	0.4876	0.4699
	M6	0.1055	0.0558	0.0573	0.0671	0.4534	0.4419	0.4658	0.4751
	M7	0.0754	0.0355	0.0277	0.0378	0.3300	0.3607	0.3452	0.3588
	M8	0.0760	0.0350	0.0268	0.0389	0.3297	0.3615	0.3440	0.3615
LSD at 0.05		0.0010	0.0011	0.0012	0.0008	0.0051	0.0040	0.0041	0.0032
T1	M1	0.1379	0.0676	0.0884	0.0871	0.4902	0.4889	0.5004	0.4951
	M2	0.1583	0.0923	0.1097	0.1128	0.5720	0.5880	0.5853	0.5978
	M3	0.1436	0.0724	0.0952	0.0976	0.5129	0.5084	0.5276	0.5372
	M4	0.1570	0.0883	0.1078	0.1111	0.5667	0.5720	0.5778	0.5911
	M5	0.1191	0.0569	0.0718	0.0782	0.5108	0.4462	0.5294	0.4596
	M6	0.1209	0.0537	0.0738	0.0773	0.4329	0.4333	0.4418	0.4560
	M7	0.1013	0.0392	0.0514	0.0542	0.3440	0.3756	0.3524	0.3636
	M8	0.1017	0.0393	0.0517	0.0558	0.3411	0.3760	0.3533	0.3698
T2	M1	0.0914	0.0568	0.0430	0.0467	0.4244	0.4458	0.4263	0.4533
	M2	0.1130	0.0760	0.0643	0.0672	0.5107	0.5227	0.5240	0.5356
	M3	0.0947	0.0583	0.0454	0.0457	0.4373	0.4520	0.4538	0.4493
	M4	0.1093	0.0719	0.0617	0.0614	0.4960	0.5062	0.5133	0.5124
	M5	0.0913	0.0536	0.0448	0.0433	0.4240	0.4329	0.4458	0.4400
	M6	0.0877	0.0498	0.0396	0.0417	0.4093	0.4178	0.4249	0.4333
	M7	0.0638	0.0263	0.0179	0.0176	0.3138	0.3240	0.3297	0.3382
	M8	0.0646	0.0270	0.0184	0.0183	0.3122	0.3267	0.3258	0.3400
T3	M1	0.1050	0.0743	0.0588	0.0972	0.4787	0.5160	0.5018	0.5356
	M2	0.1272	0.0978	0.0786	0.1141	0.5676	0.6098	0.5809	0.6147
	M3	0.1067	0.0767	0.0586	0.0981	0.4853	0.5253	0.5009	0.5391
	M4	0.1248	0.0920	0.0772	0.1136	0.5578	0.6000	0.5689	0.6009
	M5	0.1144	0.0616	0.0666	0.0829	0.5164	0.4649	0.5329	0.4782
	M6	0.1131	0.0572	0.0644	0.0951	0.5111	0.4476	0.5244	0.5271
	M7	0.0703	0.0483	0.0228	0.0610	0.3380	0.4120	0.3578	0.3907
	M8	0.0699	0.0450	0.0213	0.0622	0.3382	0.4100	0.3520	0.3929
T4	M1	0.0989	0.0623	0.0503	0.0528	0.4542	0.4680	0.4680	0.4778
	M2	0.1197	0.0826	0.0713	0.0746	0.5373	0.5489	0.5520	0.5649
	M3	0.1010	0.0631	0.0523	0.0547	0.4627	0.4711	0.4760	0.4853
	M4	0.1171	0.0796	0.0701	0.0733	0.5104	0.5369	0.5472	0.5600
	M5	0.1058	0.0663	0.0589	0.0588	0.4818	0.4840	0.4422	0.5018
	M6	0.1004	0.0626	0.0513	0.0543	0.4604	0.4689	0.4720	0.4840
	M7	0.0663	0.0281	0.0186	0.0184	0.3240	0.3311	0.3409	0.3430
	M8	0.0677	0.0287	0.0196	0.0194	0.3274	0.3333	0.3449	0.3434
LSD at 0.05		0.1300	0.0637	0.0812	0.0843	0.4713	0.4736	0.4835	0.4838

*** Means in the same column for each trait are the average of two independent experiments (n = 3). Values significantly differ from each other according to LSD test at P=0.05.**

*** (T1) 100 % Recommended dose of NPK (100:50:50 Kg/ha), (T2) 100% Recommended dose of Biogas Slurry (BGS) (6000 kg/ha), (T3) 50% NPK+ BGS (5000 kg/ha), (T4) 50 % NPK + BGS (3000 kg /ha), (M1) Azolla aqueous extract (50%) as a foliar spray, (M2) Azolla aqueous extract (50%) as drench addition, (M3) garlic aqueous extract (10%) as a foliar spray, (M4) garlic aqueous extract (10%) as drench addition, (M5) yucca aqueous extract (0.3 g/l) as a foliar spray, (M6) yucca aqueous extract (0.3 g/l) as drench addition, (M7) distilled water as a foliar spray, (M8) distilled water as drench addition.**

chlorophylls content of both seasons. Even though the second cut of both seasons took the same line with the first cut whereas Azolla aqueous extract used as drench addition with 50% NPK +5000 kg h^{-1} biogas fertilizer ($T_3 \times M_2$). In contrast, the lowest values of carotenoids along with total chlorophylls were registered by recommended dose of biogas fertilizer with distilled water as a foliar spray ($T_2 \times M_7$) of both cuts and seasons.

Stevioside and rebaudioside A., content:

Stevioside and rebaudioside A., (steviol glycoside) are the main glycosides that are responsible for the quality of stevia. Their concentration in the plant determines the sweetness and bitterness. A perusal of data presented in Table (14) and Figs. (1-4) shows that biogas fertilizer as organic manure and natural extracts of stevia recorded a significant effect on stevioside concentration as the highest values of stevioside was recorded by 50% NPK+3000 kg h^{-1} combining with Azolla aqueous extract as drench addition ($T_4 \times M_2$) followed by 50% NPK+3000 kg h^{-1} (T_4M_6) as well as recommended dose of chemical fertilizer with Azolla aqueous extract as a drench addition ($T_1 \times M_2$).

On the other hand, the highest value of both total steviol glycoside (stevioside and rebaudioside A., was recorded by 50% NPK+3000 kg h^{-1} combining with Azolla aqueous extract as drench addition ($T_4 \times M_2$) followed by recommended dose of chemical fertilizer with Azolla aqueous extract as a drench addition ($T_1 \times M_2$) while the interaction treatments of 50% NPK+ BGS (5000 kg ha^{-1}) + Azolla extract as drench addition ($T_3 \times M_2$) ranked the third in this concern.

Meanwhile, as data presented in the same Table showed that rebaudioside A., registered the highest values with the interaction between the recommended dose of biogas fertilizer and yucca aqueous extract as a foliar spray ($T_2 \times M_5$) followed by the interaction between the recommended dose of biogas fertilizer and Azolla aqueous extract as a drench addition ($T_2 \times M_2$) followed by the interaction between the recommended dose of biogas fertilizer and Azolla aqueous extract as a drench addition ($T_2 \times M_4$).

DISCUSSION

Biogas-slurry is a great soil conditioner because it adds humus and increased the organic matter, available phosphorus, and exchangeable potassium content of the soil, as well as its porosity and water holding capacity. It also reduces soil temperature fluctuations, decreases soil water evaporation, and influence on the levels of certain nutrients measured in plants (Mdlambuzi *et al*., 2021). Chemical fertilizers add more nutrients to the soil than organic fertilizers, but they can only deliver certain nutrients to the crops without enhancing soil production. However chemical fertilizers are prohibitively expensive for small-scale farmers. (Yamika *et al*., 2019) Moreover, biogas slurry lowers the cost of natural organic fertilizer while increasing soil fertility Because the physical conditions of the soil have been altered, nutrient uptake by plants has increased, resulting in increased crop yield**.** (Yan *et al*., 2021)

The reason for plant's shoots fresh and dry yield grows and gave the highest values only for the first cut as a result of the use of yucca aqueous extract as a foliar spray with chemical fertilizer, as yucca extract works to break the surface tension of the leaf because it contains steroidal saponins, allowing for more efficient gas exchange, which increases the plant's ability to perform photosynthesis and water and food absorption (Andreuccetti *et al*., 2011). This study's finding is coincided with those reported by (Jonas, 1969)**,** who found that the application of aqueous saponin-rich extracts of *Digitalis purpurea* stimulated the formation of adventitious roots on tomato cuttings, whereas application of the pure saponin, digitoxin, had the opposite effect, suggesting that digitoxin is toxic to tomato plants.

Table 14. LC-MS chromatograms of *Stevia rebaudiana* L. leaves in the first cut of the second season (2020).

Treatments	Stev.*	Reb A**	Total Stev. + RebA
T1M1: 100 % RD of NPK +Azolla ex.. as a foliar spray	9.53	0.13	9.66
T1M2: 100 % RD of NPK+Azolla ex. as drench addition	9.76	0.27	10.03
T1M3: 100 % RD of NPK+ garlic ex. as a foliar spray	9.35	0.28	9.63
T1M4: 100 % RD of NPK+ garlic ex. as drench addition	9.45	0.11	9.56
T1M5: 100 % RD of NPK+ yucca ex. as a foliar spray	9.25	0.20	9.45
T_1M6: 100 % RD of NPK+ yucca ex. as drench addition	9.62	0.12	9.74
T1M7: 100 % RD of NPK+ distilled water as a foliar spray	9.18	0.18	9.36
T1M8: 100 % RD of NPK+ distilled water as drench addition	9.12	0.09	9.21
T2M1: 100 % RD of BGS (6000 kg/ha) + Azolla extract as a foliar spray	4.22	1.10	5.32
T2M2: 100 % RD of BGS (6000 kg/ha) +Azolla extract as drench addition	4.56	2.00	6.56
T2M3: 100 % RD of BGS (6000 kg/ha) + garlic extract as a foliar spray	2.71	0.54	3.25
T2M4: 100 % RD of BGS (6000 kg/ha) + garlic extract as drench addition	1.75	1.65	3.40
T2M5: 100 % RD of BGS (6000 kg/ha) + yucca extract as foliar spray	2.04	2.79	4.83
T2M6: 100 % RD of BGS (6000 kg/ha) +yucca extract as drench addition	2.28	1.46	3.73
T2M7: 100% RD of BGS (6000 kg/ha) + distilled water as foliar spray	1.93	0.41	2.34
T2M8: 100 % RD of BGS (6000 kg/ha) + distilled water as drench addition	2.02	0.45	2.47
T3M1: 50% NPK+ BGS (5000 kg/ha) +Azolla extract as foliar spray	4.59	0.51	5.09
T3M2: 50% NPK+ BGS (5000 kg/ha) +Azolla extract as drench addition	9.58	0.34	9.92
T3M3: 50% NPK+ BGS (5000 kg/ha) +garlic extract as foliar spray	6.12	1.36	7.48
T3M4: 50% NPK+ BGS (5000 kg/ha) + garlic extract as drench addition	9.10	0.78	9.89
T3M5: 50% NPK+ BGS (5000 kg/ha) + yucca extract as foliar spray	6.64	0.06	6.70
T3M6: 50% NPK+ BGS (5000 kg/ha) + yucca extract as drench addition	9.32	0.57	9.89
T3M7: 50% NPK+ BGS (5000 kg/ha) + distilled water as foliar spray	4.12	0.11	4.23
T3M8: 50% NPK+ BGS (5000kg/ha) + distilled water as drench addition	4.12	0.11	4.23
T4M1: 50% NPK+ BGS (3000 kg/ha) +Azolla extract as foliar spray	9.43	0.10	9.53
T4M2: 50% NPK+ BGS (3000 kg/ha) +Azolla extract as drench addition	9.83	0.54	10.37
T4M3: 50% NPK+ BGS (3000 kg/ha) +garlic extract as foliar spray	9.09	0.75	9.84
T4M4: 50% NPK+ BGS (3000 kg/ha) + garlic extract as drench addition	9.49	0.13	9.62
T4M5: 50% NPK+ BGS (3000 kg/ha) + yucca extract as foliar spray	8.57	0.22	8.79
T4M6: 50% NPK+ BGS (3000 kg/ha) +yucca extract as drench addition	9.76	0.12	9.88
T4M7: 50% NPK+ BGS (3000 kg/ha) + distilled water as foliar spray	6.72	0.06	6.78
T4M8: 50% NPK+ BGS (3000 kg/ha) + distilled water as drench addition	6.95	0.09	7.04

*** Stevioside**
**** Rebaudioside A.**

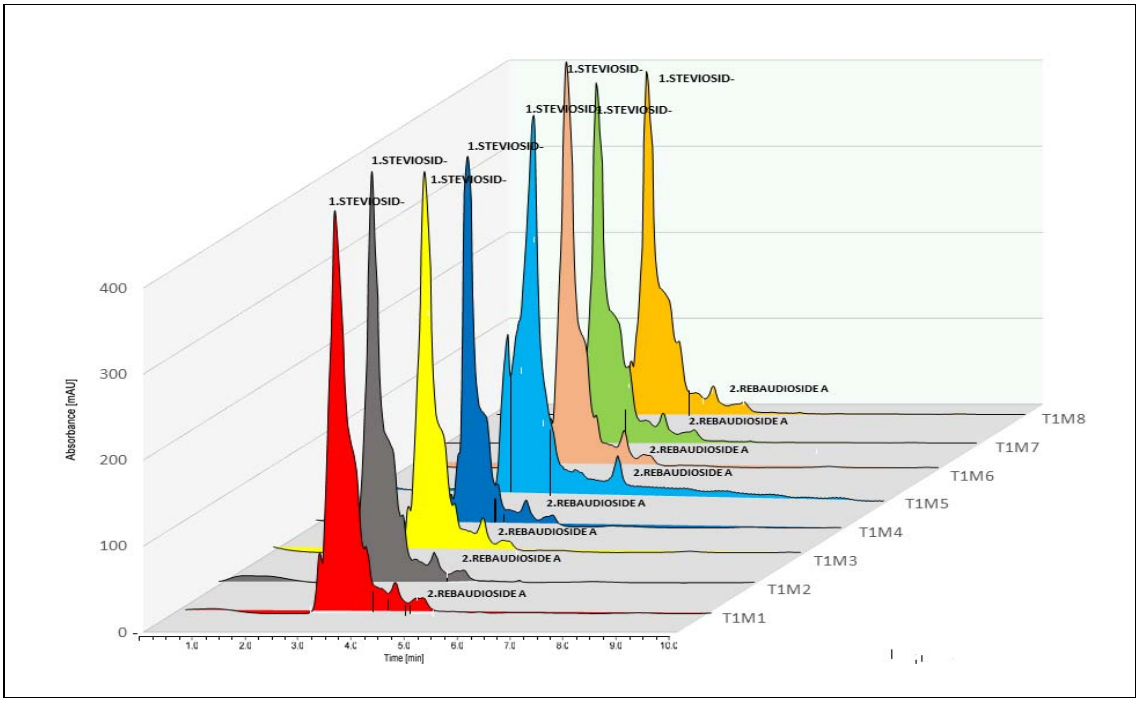

Fig. 1. LC-MS the effect of chemical fertilizer and natural extracts method application on Stevioside and rebaudioside A., chromatogram of *Stevia rebaudiana* leaves in the first cut of the second season (2020).

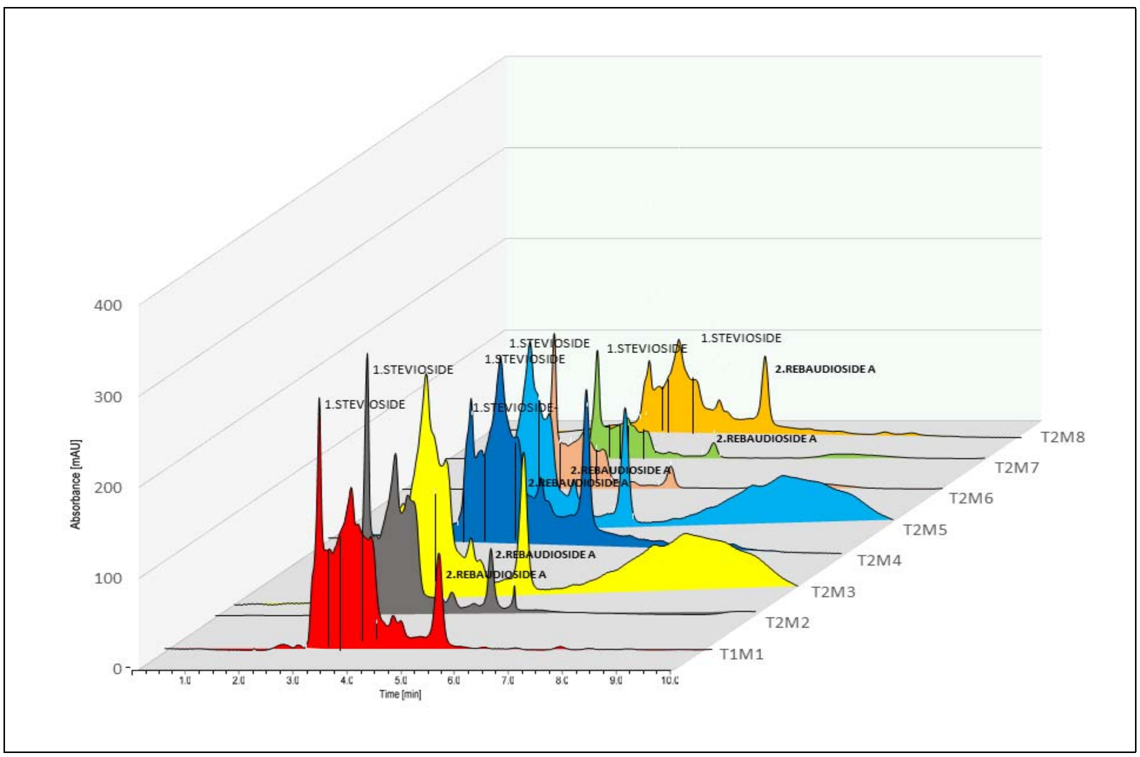

Fig. 2. LC-MS the effect of Biogas fertilizer and natural extracts method application on Stevioside and rebaudioside A., chromatogram of *Stevia rebaudiana* leaves in the first cut of the second season (2020).

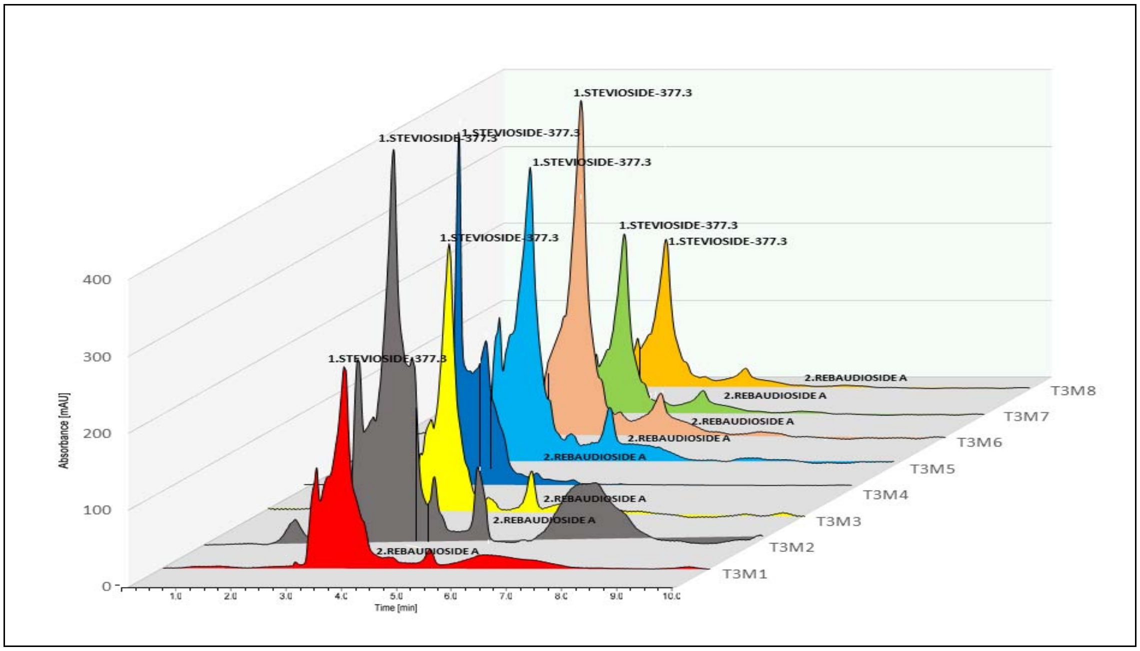

Fig. 3. LC-MS the effect of 50% NPK+5000 kg biogas and natural extracts method application on Stevioside and rebaudioside A., chromatogram of *Stevia rebaudiana* leaves in the first cut of the second season (2020).

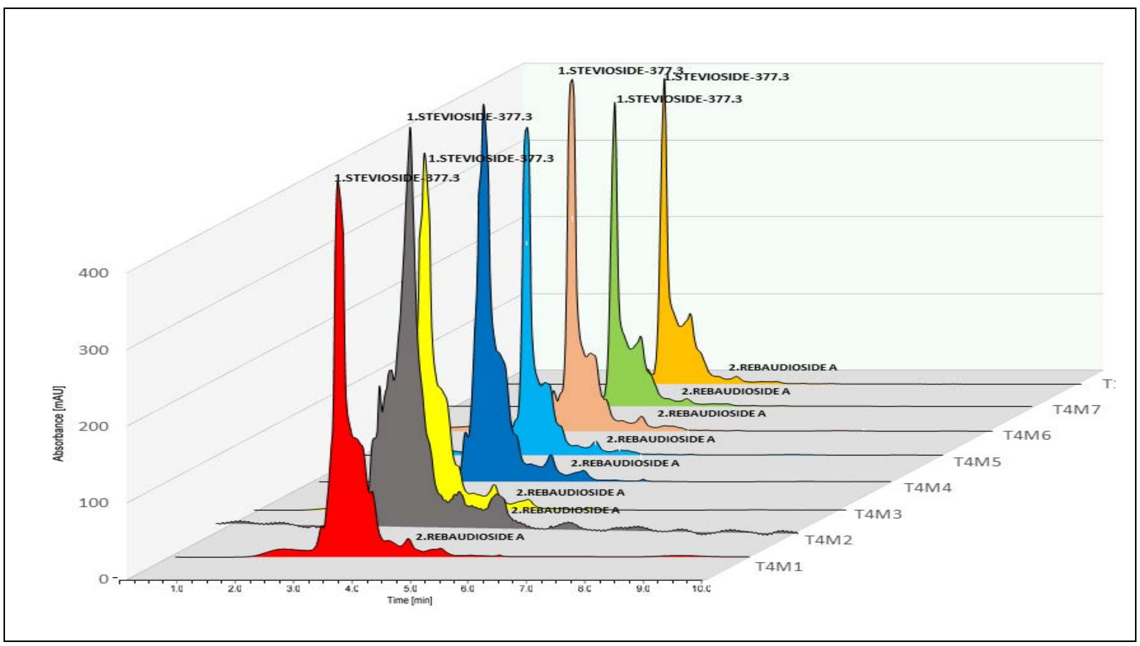

Fig. 4. LC-MS the effect of 50% NPK+3000 kg biogas and natural extracts method application on Stevioside and rebaudioside A., chromatogram of *Stevia rebaudiana* leaves in the first cut of the second season (2020).

In addition to improving soil quality, they supplied micro and macronutrients to the soil, increased nutrient uptake by plants, and increased plant growth. This was observed in biogas slurry application due to the supply of more readily available nutrients and the wider C:N ratio of biogas slurry. That is why biogas slurry generally performs better in the later stages of its application in the soil in terms of nutrient availability, as it has a more consistent effect on nutrient uptake by plants due to its mineralization occurring later in the process and slowly release nutrients rather than providing nutrients to the plants in conjunction with mineral fertilizer This explains why better results are obtained in most cases with the second cut.

On the other hand combining, biogas slurry, a half dose of the chemical fertilizer with garlic extracts gave the highest values on the shoots fresh and dry yield in the second cut as a result of an increase in number of branches, number of leaves, the leaves surface area as this may be due to the biochemical function of vitamins and amino acids in the garlic aqueous extract that improves the role of metabolic processes and endogenous hormones (IAA and GA_3) (Elzaawely *et al*., 2018). Extract of garlic resulted in cell propagation, cell expansion, and division of cells, contributing to an increase in the number of leaves. These effects can also be caused by the impact on the growth of the endogenous hormones all in treated plants of garlic extract; (Abd-Allah *et al*., 2021). These observations corresponded to (Ziedan and Eisa, 2016) on dill plant who concluded that using garlic extract with micronutrients to have better yield from fruit, it is easily available, environmentally safe and cost-effective, (Massoud *et al*., 2017) on *Majorana hortensis* L. (Massoud *et al*., 2019) on Caraway and (AbdelKader *et al*., 2014), on *Salvia officinalis* L.

Also the increases of photosynthetic pigment content, maybe due to a higher soil and nutrient retention and supply. This increase in photosynthetic pigment creation could also be a result of Azolla cytokinins' ability to prolong leaf aging by inhibiting chlorophyll breakdown and promoting protein and RNA synthesis (Castelfranco and Beale, 1983). These findings also corroborated with (Vanithamani, 2016) who stated that the greatest impact was exercised in organic fertilizer treatment (vermicompost) and half dose of NPK + Azolla. treatment revealed an increase in the photosynthetic pigments of chlorophyll (chlorophyll a, b and total chlorophylls) and carotenoids compared to untreated plants in *Amaranthus polygonoids* L.

Biogas slurry also contains organic nitrogen (primarily amino acids), abundant mineral elements, and low-molecular-mass bioactive substances (e.g. hormones, humic acids, vitamins, etc.) and may be due to bioactive compounds such as auxin, cytokinin, and gibberellins could be extracted from garlic and Azolla extracts as shown in this study which was used as an exogenous growth regulator. Whereas increased mineral content such as N.P.K may be due to the highest gibberellin (GA_3), auxin (IAA), and cytokinins (zeatin) contained in garlic and Azolla extract.

In this concern, the result is supported by the study of (Calvo *et al*., 2014), who stated that microbial inoculants could enhance nutrient uptake and thus improve the crop's nutrient status. As with fertilizers, biostimulants can help improve plant nutrition by stimulating natural mechanisms that aid in nutrient absorption and efficiency (De Pascale *et al*., 2017). (Al-karaawi, 2019) reported that administration of gibberellin (GA_3) may increase growth due to an increase in the effective leaf area hence photosynthesis increases. Cytokinins are compounds that can increase cell division in plant tissues and regulate plant growth and development, as well as kinetin, Additionally, soil-applied organic matter not only provides structure to the soil, but also provides energy for microbial activity that is necessary for nutrient recycling, affects nutrient availability such as N, P, K, and S, improves soil and water conservation, soil buffering and exchange capacity(Adebayo *et al*., 2017).

These organic fertilizers may accelerate plant growth in certain circumstances. The inclusion of growth promoters like auxin in biogas slurry is the primary rationale for this action (Warnars and Oppenoorth, 2014). Furthermore, it has been claimed that these can increase the permeability of the cell membrane, increasing water, and other nutritional element uptake. Additionally, water absorption and nitrogen content rose dramatically. Similarly, the inclusion of organic-based chemicals significantly increased root and shoot growth, as well as water uptake, in the N-free medium (Koszel and Lorencowicz, 2015).

On the other hand, the results obtained for stevia leaf nitrate concentrations indicated that while biogas slurry as an organic fertilizer decreased nitrate concentrations, the combination of biogas fertilizer and Azolla reduced nitrate concentrations to levels lower than those obtained with biogas alone in two seasons (Kawtar *et al*., 2017). It's worth noting that the nitrate levels observed in all treatments investigated are within regulatory limits. The former Scientific Committee on Food (SCF) established an average daily intake (ADI) for nitrate of 3.7 mg/kg body weight per day, equivalent to 222 mg nitrate per day for a 60 kg adult, which was reconfirmed in 2002 by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) and (E.F.S.A., 2008). Nitrate is benign at levels below the maximum residue levels (MRLs), but when it exceeds these levels, it can be harmful due to the decrease of nitrites, which can combine with amines and amides to form "N-nitroso" compounds that cause gastric cancer (SeyyedSalehi *et al*., 2021). These results agreed with (Rabie *et al*., 2020) on *Matricaria Chamomilla* who concluded that the addition of compost and 50% of chemical fertilizer to Azolla extract at 50% as foliar (spray Azolla) had a positive effect on chemical constituents except nitrate and nitrite concentrations. Meanwhile, (Fallah and Omrani, 2018) demonstrated that while nitrate concentrations in plants increased significantly following nitrogen fertilizer application, organic fertilizer use can lower nitrate concentrations in medicinal plants.

The glycosides accumulation of stevia leaves takes place throughout the life of the *Stevia rebaudiana* L., especially in the later stage of growth. At the latter growth stage, there is a large leaf area and a high net photosynthetic rate, which result in a significant buildup of photosynthesis product (Liu *et al*., 2011). Each physiological index is much higher in organic manure cultivation than in chemical fertilizer cultivation (Enchev *et al*., 2018). Rebaudioside A., has a weaker off-flavor than stevioside (Jung *et al*., 2021). Steviol glycosides with a superior taste are being investigated to generate sweeteners with a sensory profile that is closer to sucrose. The industry also uses rebaudioside A., which is now widely used (Rashwan and Ferweez, 2017). The ratio of rebaudioside A., to stevioside is the accepted measure of sweetness quality; the more rebaudioside A., the better. If rebaudioside A., is present in equal quantities to stevioside, it appears that the after taste is eliminated (Majzoobi *et al*., 2018) To summarise, organic manure culture can enhance above-ground growth, root vigor, leaf net photosynthetic capacity, and total glycosides content in the leaf of Stevia. Thus, employing biogas fertilizer sparingly can improve the taste as reduces the sense of bitter taste. and quality of *Stevia rebaudiana* Bertoni, as well as improve the soil structure and safeguard the environment.

The results were in agreement with (Zaman *et al*., 2018) (Liu *et al*., 2011) (Rashwan and Ferweez, 2017) where the use of organic fertilizer improved the productivity and quality of stevia plants compared to chemical fertilizer, either by using organic fertilizer with half the dose of chemical fertilizer, which significantly affected the plant content of both stevioside and rebaudioside A., which led to obtaining the highest percentage of stevioside and the total glycosides. The increase in the total content of stevioside and rebaudioside may be due to the use of Azolla extract, which led to an increase in the leaf area, including enhancing the utilization of light energy and increasing the plant's chlorophyll content (Maswada *et al*., 2021) and enhancing the process of photosynthesis, which enhances the production of stevioside and rebaudioside**.[**(Sadegh Kasmaei *et al*., 2019)**;** (Paler and Alcantara, 2021)**]**.

CONCLUSION

Biogas used as organic fertilizer is characterized by an increase in nitrogen content, which improves the plant's chemical constituents, hence, increased yield and quality but it requires time for analysis, which were evident in the second cut of both seasons. The results indicated that substituting half of the recommended dose of chemical fertilizer with the same equivalent dose from the nitrogen content for biogas fertilizer resulted in a reduction of the problem of chemical fertilizer on human health included in nitrate accumulation and economic damages, in addition to the use of both Azolla and garlic extracts as a drench additive with organic fertilizer.

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استجابة نبات الاستيفيا لسماد البيوجاز والمستخلصات الطبيعية تحت ظروف جنوب سيناء

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أجريت تجربتن حقليتين في موسمي٢٠١٩ و ٢٠٢٠ بمركز بحوث الصحراء بالمطرية بالقاهرة مصر ومزرعتها التجريبية بمحطة رأس سدر التجريبية بجنوب سيناء بالتعاون مع قسم البساتين بكلية الزراعة. جامعة بنها، لدراسة تأثير سماد البيوجاز والمستخلصات الطبيعية على المحصول والمكونات الكيميائية والجودة لنبات الإستيفيا ريبوديانا. أظهرت النتائج أن سماد البيوجاز مع المستخلصات الطبيعية المختلفة كان لهما تأثير معنوي على المحصول ، والمكونات الكيميائية،

و Stevioside، ,.rebaudioside A لنبات الاستيفيا. كانت أعلى القيم في معظم القراءات ، الوزن الطازج والجاف ، N، P، K ، الكلورفيل في الحشة الأولي بمعاملة الجرعة الموصى بها من السماد الكيميائي (T_1) مع (M_4) أو (M_5)) أو (M_2). من ناحية أخرى ، فإن الحشة الثانية أخذت نفس الاتجاه مع الحشة الأولي ولكن مع (T_3) 50٪ NPK +5000 kg ha^{-1} مثل ($T_3 \times M_4$) أو ($T_3 \times M_2$) اما مستخلص الثوم أو مستخلص الأزولا كأضافة ارضية سجلت الإضافة أعلى قيم للمعلملامات المذكورة أعلاه في معظم الحالات. بالنسبة لأعلى قيم لمحتوى الستيفيوسيد ومحتوى الستيفيوسيد الكلي المسجل بواسطة 50٪ NPK +3000 kg ha^{-1} ($T_4 \times M_2$) مع المستخلص المائي للازولا كإضافة ارضية وكانت نتائج rebaudioside A اعلى مع معاملة المزيج من الجرعة الموصى بها من سماد البيوجاز مع مستخلص مائي لليوكا كرذاذ ورقي ($T_2 \times M_5$). وقد اوضحت النتائج بشكل قاطع أن استخدام ٥٠٪ من الجرعة الموصى بها من السماد الكيماوي بما يعادل محتوى النيتروجين الموصى به واستبدالها بأسمدة الكيماوى بالإضافة إلى استخدام كلا من مستخلصي الآزولا والثوم كإضافة ارضية ادت إلى تقليل تراكم النترات حيث مشكلة استخدام الأسمدة الكيماوية لما لها من أضرار صحية واقتصادية.