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SYNERGISTIC EFFECTS OF BIOGAS FERTILIZER AND NATURAL EXTRACTS OF GARLIC, AZOLLA, AND YUCCA ON THE VEGETATIVE GROWTH AND ESSENTIAL OIL YIELD OF *STEVIA REBAUDIANA* L. PLANT UNDER SOUTH SINAI CONDITIONS

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ABSTRACT: Two field experiments were conducted in 2018/2019 and 2019/2020 seasons, at Ras Sidr Experimental Station in south Sinai Affiliated to Desert Research Center, Mataria, Cairo Egypt in cooperation with the Department of Horticulture, Faculty of Agriculture, Benha University, to study the effect of biogas fertilizer and natural extracts on vegetative characteristics and essential oil yield of *Stevia rebaudiana* L. plant. Results showed that biogas fertilizer along with different natural extracts exerted a significant influence on the growth characteristics and essential oil content of stevia. The highest values in most of the parameters in the first cut were found from T_1 (recommended dose of chemical fertilizer) combining with (M_4), (M_5) or (M_2). On the other hand, the second cut took the same line as the first cut but with T_3 (50% NPK + biogas slurry at 5000 kg h^{-1}) as (T_3×M_4) or (T_3×M_2) as drench addition registered the highest values of parameters mention before in most cases. Conclusively, the use of half (50%) of the recommended dose of chemical fertilizer with the equivalent of the recommended nitrogen content and replaced it with biogas fertilizer led to reducing the problem of the use of chemical fertilizers on the health and economic damages, in addition to the use of both extracts of Azolla and garlic as a drench addition with organic fertilization and half the dose of chemical fertilizer.

Key words: *Stevia rebaudiana,* organic fertilizer natural extracts, essential oil, biogas fertilizer, Azolla extract, garlic extract, yucca extract.

INTRODUCTION

Stevia rebaudiana (Beit.) is a perennial, short-day herb native to Paraguay and Brazil. It is distributed widely in Brazil, Japan, Korea, Taiwan, and USA. It belongs to Asteraceae family, sugar, which is obtained from stevia, is considered to be one of the best alternative natural sources for artificial sugars such as aspartame, saccharin, and diabetics (Benhmimou *et al*., 2017)**.** Although Sugar, which is obtained from stevia, is considered to be one of the best alternative natural sources for artificial sugars such as aspartame, saccharin for diabetics and those who want to make a diet, (Esen, 2016**)**. However, Stevia also contains essential oils, which increase its therapeutic uses as high antioxidant, anti-inflammation, and antimicrobial (Muanda *et al*., 2011)**.** Numerous studies have established, certain effects of stevia essential oil on the human organism, it has become interesting for the pharmaceutical industry among the therapeutic activities attributed to its hypersensitive regulation, hypoglycemic, antimicrobial, and contraceptive can be listed, (Cioni *et al*., 2006). To our knowledge, there are only a few studies on the composition of the essential oil in stevia and the difference in its compounds as a result of the different places of production, (Ivana *et al*., 2007). Therefore, we conduct this study under the conditions of Ras Sudr city in South Sinai.

The organic cultivated area in the world is estimated at 57.8 million hectares in 2018, and that this area is increasing annually at a rate of about 4.4% during the period (2005-2018). Egypt ranks (48) in the world in terms of the area organically grown, with about 105,908 hectares in 2018, and the area has organically grown in Egypt amounted to about 2.8% of the total area organically grown in the world (Willer *et al*., 2019).

Biogas and post-digestion matter are produced, which can be used as fertilizers in agriculture. Substrates for the Methane fermentation require organic waste from agriculture, the food industry, or urban greenery conservation, and also Sludge from the care of sewage. (Gis and Samson-bręk, 2012). A biogas digester not only provides clean and cheap energy but also produces a good organic fertilizer for crops. Indeed, because of its high nutrient content, bio-slurry is an excellent organic fertilizer and alternative for farmyard manure, complementary to normal compost and sometimes even to chemical fertilizers, (Groot and Bogdanski, 2013)

It's been strong recently, voices for the exploitation of natural compounds to conserve the setting and avoid the side effects where they exist Specialists focus on plant extracts as an alternative Materials for synthetic chemicals (Abd El–Hamied and El-Amary, 2015). Plant extracts such as garlic, Azolla, and yucca extract have been registered as an efficient means of production Supply plants with the requisite nutrients to the foliage sections are faster than the root system (Abu *et al*., 1988). The extract of garlic is enriched by nutrients Where 31 percent carbohydrate was found in the bulbs, 6.2 percent Phosphors, iron, potassium, as well as proteins, Magnesium, and vitamins such as thiamine, niacin, riboflavin, and ascorbic acid. Amino acids contained Sulphur such as cysteine, and methionine were also present in the bulb. Derivative-Allicin cysteine (Abdulrazzaq, 2017).

Azolla is a small aquatic fern that can grow without combined nitrogen due to nitrogen fixation by symbiotic cyanobacteria and it has been used as green manure or foliar spray for many crops (Watanabe *et al*., 1991). It is well established that freshly separated *Anabaena azolla* releases about 40-50% of nitrogen fixed as ammonia into the immediate environment (Meeks *et al*., 1987). Azolla is used as a nitrogen supplement in rice-based ecosystems, it has also been used in other crops such as taro, wheat, tomato, and banana. The incorporation of fresh fronds of Azolla also increased the grain yield of wheat (Marwaha *et al*., 1992).

Yucca is rich in natural moisturizing factors called saponins. Saponins are natural steroid-based chemicals that make water more moisture, which breaks the polarity of the water molecule (Wulff *et al*., 2012). Using yucca extracts regularly can improve water and mineral absorption, and prevent many soil problems from developing. Yucca in irrigation water can help keep dripping emitters clean. In response to desert conditions, yucca produces powerful antioxidants that protect delicate cell membranes from damaging free radicals. Some antioxidants, such as salicylic acid and vitamin C, have a direct protective effect on plants. But yucca also produces signaling molecules that stimulate plants to make more of their natural plant protectants. (Rachael, 2018).

This study aimed to improve the vegetative growth and essential oil yield of *Stevia rebaudiana* L. plant. by using biogas fertilizer and natural extracts under Ras Sudr city in South Sinai conditions.

MATERIALS AND METHODS

Site description:

Two field experiments were conducted in 2018/2019 and 2019/2020 season, 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 vegetative characteristics and essential oil yield of *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 2018/2019 and 2019/2020. The physical and chemical properties of the soil sample were determined, according to (Rathje, 1959) (Cottenie *et al*., 1982), and are shown in Tables (1 and 2). Drip irrigation lines were installed within 20 cm between drippers with an average of 4 l/h. Underground water was used as an irrigation source and 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 under the full daylight hours. The experimental plot was 5.85 m^2 (20 × 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).

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.

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

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 a 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 extract was prepared according to (Elzaawely *et al*., 2018) 100 g of freshly grown cloves of garlic (*Allium sativum*) 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 (-20º C). 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).

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	**GA3**	113.72 ng ml^{-1} fw
	GA4	68.53 ng ml^{-1} fw
	GA7	173.52 ng ml^{-1} fw

Azolla extract:

Azolla was obtained from the Land and Water Research Institute, Agriculture Research Center. 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. The plant material is 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 Shima *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 and the chemical composition of yucca extract is shown in Table (7).

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

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

Growth analysis:

Stevia was cut twice in each season of the study. The first cut was conducted on July, 15th while the second was done on October 15th. In each cut data on plant height (cm), number of branches per plant, and number of leaves/plants were recorded. After harvest, the plants were removed to estimate the fresh weight of both stems and leaves (gm) and leaf area (cm^2). Samples of the plants were air-dried under shade to determine the dry weight in both stems and leaves (g).

Determination of volatile oil:

According to (Egyptian Pharmacopoeia, 2005). The essential oil was determined in the dried shoots by extracting using the hydrodistillation process by the Clevinger apparatus. The obtained volatile oils samples were dried and kept in a refrigerator (Guenther, 1961). The essential oil samples obtained from the first cut of the second season were subjected to GC/ El-MS analysis,

Statistical analysis:

The means of all obtained data from the studied factors were subjected to analysis of variance (ANOVA) as factorial experiments in a split-plot design. Biogas and chemical fertilizer levels treatments (T) were assigned in main plots, which were split into eight treatments of natural extracts application methods. Collected data for both seasons were pooled and the obtained results were analyzed using the MSTATC program. Means were compared using LSD test at 0.05 level according to (Snedecor and Cochran, 1989).

RESULTS

Plant height and number of branches:

Data presented in Table (8) illustrated that all fertilization treatments succeeded in increasing plant height and number of branches of *Stevia rebaudiana* plant, particularly T_1 (recommended dose of chemical fertilizer) in the first cut in both seasons. In the second cut in both seasons T_3 (50% NPK + 5000 kg h^{-1} biogas fertilizer) score the highest values of both parameters. In the contrast, the lowest values of plant height and number of branches are registered by T_2 (100 % recommended dose of biogas fertilizer) in both cuts and seasons.

As for natural extracts treatments, data in the same Table declared that the tallest plants and the greatest number of branches were scored by garlic aqueous extract as drench addition at 10% (M_4) of the two cuts as well as 1st and 2nd seasons. while the lowest values were recorded by distilled water as a drench or foliar application method (T_7 and T_8) without significant differences between them.

Additionally, the interaction between the recommended dose of chemical fertilizer and yucca aqueous extract as a foliar spray $T_1 \times M_5$ gave the tallest plant height for both cuts in both seasons followed by $T_3 \times M_4$ (50% NPK + 5000 kg h^{-1} biogas combining with garlic aqueous extract as a drench addition) with non-significant differences between them in the second cut only. Furthermore, the highest number of branches was recorded by the combination between $T_1 \times M_4$ recommended dose of chemical fertilizer and garlic aqueous extract as a drench addition in the first cut in the two seasons. In the second cut in both seasons the interaction between 50% NPK + 5000 kg h^{-1} biogas and garlic aqueous extract as drench addition ($T_3 \times M_4$) gave the highest number of branches. On the reverse, the shortest plants were scored by the interaction between the recommended dose of biogas fertilizer with distilled water as drench addition ($T_2 \times M_8$) also the same trend of results was obtained with a number of branches but with distilled water as a foliar spray in both cuts of the both seasons.

Number of leaves/plant and leaf area:

Concerning the response of the number of leaves per plant and leaf area (cm^2) to the different investigated fertilization treatments, data presented in Table (9) show that the same trend previously discussed with number of branches was also detected in number of leaves and leaf area, recommended dose of chemical fertilizer (T_1) recorded the highest values in the first cut only in both seasons. However, the treatment of 50% NPK + 5000 kg h^{-1} biogas fertilizer (T_3) scored the highest values of these parameters in the second cut in both seasons.

Regarding natural extracts treatments, data showed that plants treated with garlic aqueous extract as a drench addition at 10% (M_4) gave the highest number of leaves of two cuts as well as both seasons concerning leaf area which did not take the same trend with number of leaves as the highest leaf area values were recorded by Azolla aqueous extract at 50% (M_2) in both cuts of both seasons. while distilled water as a drench or foliar application method also T_7 and T_8 recorded the lowest values without major variations between them.

Table 8. Effect of biogas, chemical fertilizer, and natural extracts and their interaction on plant height and number of branches per plant of *Stevia rebaudiana* L. plants in 2019 and 2020 seasons for two cuts.

Fertilization	Natural ex. Methods app	Plant height (cm)				No. of branches/plant			
		2019		2020		2019		2020	
		1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut
T1		48.51	48.61	43.95	46.89	20.85	30.92	19.75	29.29
T2		28.99	29.63	27.04	29.64	5.58	10.88	5.71	11.17
T3		44.84	47.55	42.81	46.84	17.25	31.13	17.92	30.52
T4		41.23	39.92	37.77	41.20	14.58	21.08	15.08	21.21
LSD at 0.05		1.14	0.77	1.32	0.34	1.75	1.37	0.96	0.81
	M1	40.65	42.46	37.37	40.22	13.42	22.17	14.42	24.25
	M2	43.08	47.47	38.77	46.58	16.92	27.67	17.33	28.29
	M3	44.24	43.41	39.51	44.16	14.83	24.75	16.75	25.67
	M4	45.98	48.23	42.59	47.57	19.77	29.08	18.25	30.25
	M5	45.26	45.03	42.47	44.25	14.83	22.50	13.33	20.92
	M6	40.35	37.88	38.19	39.44	13.75	21.67	13.42	21.58
	M7	34.22	33.59	32.52	33.52	11.25	20.17	11.08	16.00
	M8	33.38	33.32	31.73	33.39	11.75	20.00	12.33	17.42
LSD at 0.05		1.12	0.83	1.05	0.66	1.57	1.30	1.69	1.33
T1	M1	51.43	47.78	46.11	47.75	20.00	31.33	18.67	31.33
	M2	44.22	51.47	37.56	50.27	25.33	35.33	23.33	34.67
	M3	54.56	50.14	46.11	48.09	22.67	32.33	23.00	31.00
	M4	45.55	53.49	41.67	51.48	28.78	37.00	25.67	36.33
	M5	59.89	57.96	53.67	55.53	20.67	29.33	18.00	24.67
	M6	47.53	44.43	41.33	44.41	18.67	31.33	17.00	26.67
	M7	43.21	41.79	42.62	38.77	16.33	25.00	15.00	24.33
	M8	41.72	41.79	42.56	38.78	14.33	25.67	17.33	25.33
T2	M1	26.11	31.74	22.83	25.64	5.00	8.33	4.67	7.33
	M2	29.56	36.00	29.09	34.89	5.33	12.00	6.67	12.33
	M3	31.28	31.89	30.33	34.21	6.00	12.00	7.00	12.00
	M4	36.58	35.16	33.83	35.46	5.00	13.00	5.67	14.67
	M5	36.58	32.67	34.78	32.61	7.67	11.67	5.67	11.67
	M6	30.22	29.33	31.50	31.83	5.67	12.00	5.00	11.00
	M7	21.11	20.63	17.55	21.46	4.00	8.33	5.00	10.00
	M8	20.49	19.58	16.38	20.98	6.00	9.67	6.00	10.33
T3	M1	43.78	47.90	41.25	45.93	16.33	32.33	19.00	36.67
	M2	50.78	55.38	47.67	53.68	22.00	38.00	22.67	39.50
	M3	45.78	47.53	44.50	51.12	17.00	33.33	23.00	36.00
	M4	56.35	56.75	52.50	54.36	25.97	39.33	24.67	42.00
	M5	42.78	50.62	41.69	47.52	15.33	25.33	14.00	29.33
	M6	41.98	42.29	42.24	43.97	17.00	23.33	15.33	27.00
	M7	39.48	39.75	36.78	39.00	12.00	30.33	12.33	15.67
	M8	37.81	40.14	35.89	39.19	12.33	27.00	12.33	18.00
T4	M1	41.28	42.43	39.30	41.54	12.33	16.67	15.33	21.67
	M2	47.78	47.03	40.78	47.48	15.00	25.33	16.67	26.67
	M3	45.33	44.09	37.11	43.23	13.67	21.33	14.00	23.67
	M4	45.44	47.50	41.89	48.98	19.33	27.00	17.00	28.00
	M5	41.78	38.88	40.22	41.35	15.67	23.67	15.67	18.00
	M6	41.67	35.48	37.67	37.55	13.67	20.00	16.33	21.67
	M7	33.08	32.18	33.12	34.84	12.67	17.00	12.00	14.00
	M8	33.51	31.78	32.10	34.62	14.33	17.67	13.67	16.00
LSD at 0.05		2.24	1.67	2.11	1.33	3.14	2.60	3.37	2.67

*** 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 number of leaves and leaf area of *Stevia rebaudiana* L. plants in 2019 and 2020 seasons for two cuts.

Fertilization	Natural ex. Methods app	No. of leaves				Leaf area cm^2			
		2019		2020		2019		2020	
		1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut
T1		349.53	501.82	315.89	443.91	7.55	6.97	7.12	6.15
T2		82.36	164.47	78.18	161.86	5.13	4.50	5.03	4.40
T3		295.75	508.74	264.48	479.71	7.49	7.07	6.97	6.20
T4		199.93	278.73	191.82	298.34	6.48	6.49	5.85	5.13
LSD at 0.05		23.03	16.21	13.202	12.85	0.18	0.08	0.06	0.09
	M1	227.48	333.63	200.90	352.13	7.08	7.29	6.87	6.15
	M2	295.34	447.20	281.94	455.55	7.96	7.58	7.72	6.56
	M3	276.41	393.36	246.32	406.81	6.93	7.03	6.55	5.87
	M4	322.79	512.66	292.80	509.16	7.53	7.36	7.51	6.29
	M5	243.66	392.37	211.23	341.82	6.52	5.88	5.75	5.20
	M6	202.01	339.18	186.49	301.82	6.35	5.81	5.53	5.07
	M7	143.46	246.30	134.29	191.30	5.41	4.60	5.07	4.30
	M8	143.98	242.82	146.79	209.04	5.50	4.50	4.95	4.29
LSD at 0.05		23.30	21.97	22.90	20.62	0.17	0.07	0.08	0.07
T1	M1	343.38	499.15	288.67	453.26	8.29	7.96	8.12	6.86
	M2	429.00	560.39	405.67	534.91	8.70	8.20	8.33	7.60
	M3	412.07	539.78	362.51	467.94	8.14	7.64	7.82	6.64
	M4	469.44	689.59	431.83	623.58	8.21	7.80	8.17	7.02
	M5	412.26	555.83	341.73	464.50	7.27	6.62	6.62	5.88
	M6	295.24	463.84	245.33	365.63	6.91	6.73	6.18	5.63
	M7	235.21	348.26	206.07	314.24	6.28	5.47	5.88	4.83
	M8	199.63	357.72	245.33	327.19	6.55	5.30	5.85	4.72
T2	M1	65.00	132.44	53.08	94.12	5.64	5.14	5.35	5.00
	M2	79.06	216.11	96.87	196.93	6.11	5.83	6.05	5.35
	M3	93.81	191.94	106.77	204.89	5.47	4.83	5.21	4.73
	M4	91.55	228.37	95.87	259.92	5.93	5.57	5.87	5.12
	M5	140.11	190.24	98.06	190.48	5.24	4.20	4.97	4.28
	M6	85.94	176.25	78.49	175.06	5.28	4.08	4.74	4.13
	M7	42.24	86.02	47.82	84.53	3.77	3.18	4.12	3.25
	M8	61.14	94.39	48.52	88.97	3.64	3.18	3.97	3.30
T3	M1	332.23	467.55	260.99	561.34	8.10	8.14	7.82	7.17
	M2	434.33	672.00	398.04	668.44	9.15	8.21	8.53	7.62
	M3	393.33	528.04	341.33	613.36	7.84	7.95	7.37	6.90
	M4	436.94	704.94	406.67	696.00	8.23	8.19	8.28	7.53
	M5	218.14	516.55	194.61	464.10	7.13	6.75	6.24	5.33
	M6	238.24	479.96	215.81	395.40	6.85	6.70	6.15	5.24
	M7	157.96	368.34	150.63	203.81	6.27	5.32	5.73	4.93
	M8	154.82	332.52	147.74	235.21	6.32	5.30	5.62	4.84
T4	M1	169.31	235.38	200.87	299.80	6.29	7.94	6.18	5.58
	M2	238.96	340.29	227.19	421.93	7.88	8.06	7.96	5.68
	M3	206.44	313.70	174.67	341.06	6.27	7.70	5.82	5.21
	M4	293.22	427.73	236.81	457.13	7.74	7.89	7.73	5.50
	M5	204.14	306.87	210.52	248.19	6.44	5.96	5.15	5.30
	M6	188.63	236.65	206.33	271.19	6.37	5.70	5.03	5.26
	M7	138.43	182.58	132.63	162.60	5.32	4.42	4.55	4.19
	M8	160.31	186.65	145.57	184.78	5.50	4.22	4.37	4.32
LSD at 0.05		46.59	43.95	45.81	41.24	0.34	0.15	0.16	0.13

*** 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.**

As shown by the same Table, the average number of leaves was significantly increased through application of the interaction between the recommended dose of chemical fertilizer and garlic aqueous extract as a drench addition ($T_1 \times M_4$) only in the first cut in both seasons followed by ($T_3 \times M_4$) and ($T_3 \times M_2$) with non-significant differences between them. However, the second cut the statistically increase was detected by interaction between 50% NPK + 5000 kg h^{-1} and garlic aqueous extract ($T_3 \times M_4$) followed by ($T_3 \times M_2$) which recorded the highest leaf area values in the first and second cuts in both seasons.

On the other hand, the interaction between the recommended dose of biogas fertilizer with distilled water as foliar or drench addition ($T_2 \times M_7$ or $T_2 \times M_8$) scored the least effective treatments on leaf area and the number of leaves values for 1st and 2nd cuts in both seasons.

Shoots fresh and dry weights:

Data presented in Table (10) show obviously that the response of average shoots fresh and dry weights followed the same trend previously detected with the former four growth parameters as recommended dose of chemical fertilizer (T_1) in the first cut in both seasons. While the second cut took the same trend with the first cut in both seasons followed by 50% NPK +5000 kg h^{-1} (T_3) without significant variations between them. On the opposite, the lowest fresh and dry weights came from plants treated with recommended dose of biogas fertilizer in both cuts and seasons (T_2).

The obtained result regarding the increment in shoots fresh and dry weights by the different natural extracts application methods proved that garlic aqueous extract as drench addition at 10% (M_4) scored the highest values of shoots fresh weight in the first cut of both seasons followed by Azolla aqueous extract at 50% as a drench addition with non-significant differences between them in the second cut. Although the shoots dry weight goes in the line with those found in fresh weight as (M_4) have the highest dry weight values in both 1st and 2nd cut but without significant variance with (M_2) at both seasons. At the same time, the lowest values were registered by the distilled water as drench or foliar application method (T_7 and T_8) with non-significant differences between them.

Accordingly, it is quite clear to be noticed from tabulated data that the positive effect of the interaction between the recommended dose of chemical fertilizer and yucca aqueous extract as a foliar spray ($T_1 \times M_5$) gave the heaviest plant fresh and dry weights only in the first cut in both seasons. Additionally, the second cut registered the heaviest fresh and dry weights by 50% NPK +5000 kg h^{-1} biogas combining with garlic aqueous extract as drench addition ($T_3 \times M_4$) in both seasons. In contrast, the least fresh and dry weights were recorded by 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 while the second cut took the same line with the first but with distilled water as a drench addition ($T_2 \times M_8$).

Leaves fresh and dry weights:

Data presented in Table (11) declared that treatment by using recommended dose of chemical fertilizer (T1) scored the heaviest weight of the fresh and dry leaves only in the first cut of both seasons. Although the second cut recorded the highest leaves fresh weight in both seasons by 50% NPK +5000 kg h^{-1} biogas fertilizer (T_3), however, in the case of the leaves dry weight there were no significant differences with (T_1) in both seasons. whereas the least values of fresh and dry leaves weight were 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 weight to natural extracts revealed that Azolla aqueous extract (M_2) registered the highest values in both cuts and

Table 10. Effect of biogas, chemical fertilizer, and natural extracts and their interaction on shoots fresh and dry weights of *Stevia rebaudiana* L. plants in 2019 and 2020 seasons for two cuts.

Fertilization	Natural ex. Methods app	Shoots fresh weight (g/plant)				Shoots dry weight (g/plant)			
		2019		2020		2019		2020	
		1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut
T1		137.08	145.87	130.58	140.46	36.09	38.69	33.97	36.88
T2		54.89	66.50	48.77	56.97	12.14	15.52	11.20	13.21
T3		129.52	146.49	124.59	139.93	30.60	38.97	30.28	36.40
T4		119.21	139.83	112.05	122.53	29.00	32.65	28.24	30.88
LSD at 0.05		2.48	1.44	2.27	2.43	0.54	0.38	0.71	0.75
	M1	114.67	123.09	110.25	115.83	26.69	31.30	27.41	29.41
	M2	124.46	134.25	118.39	126.61	32.30	35.47	31.57	34.12
	M3	116.00	128.98	112.05	121.52	28.40	33.13	28.55	31.21
	M4	127.54	134.83	121.84	128.25	32.92	36.03	31.78	34.48
	M5	112.94	124.45	112.50	114.60	28.12	32.19	26.66	29.29
	M6	112.20	122.02	110.44	108.97	25.78	31.17	26.66	27.79
	M7	87.13	115.21	73.58	102.04	20.57	26.19	17.57	24.07
	M8	86.45	114.57	72.92	101.94	20.87	26.19	17.22	24.34
LSD at 0.05		2.36	1.92	2.50	2.27	0.74	0.58	0.63	0.56
T1	**M1**	140.74	142.63	135.20	139.13	35.89	37.08	34.75	36.07
	M2	146.30	149.57	138.43	141.81	41.63	40.83	38.67	38.57
	M3	142.56	143.97	144.93	138.99	38.33	38.58	37.89	36.77
	M4	148.48	150.30	145.94	143.79	40.54	41.33	37.38	39.54
	M5	150.50	153.29	148.15	148.97	42.14	41.42	39.71	40.38
	M6	141.97	150.83	137.37	138.95	35.49	40.08	33.74	37.70
	M7	114.10	138.52	99.14	136.76	27.61	34.75	25.38	33.01
	M8	112.02	137.83	95.51	135.26	27.11	35.47	24.26	32.97
T2	**M1**	50.00	61.26	49.74	50.66	11.50	14.64	11.64	11.81
	M2	63.89	77.53	56.94	66.99	15.33	19.15	13.72	17.30
	M3	58.64	73.51	48.52	65.85	13.19	17.64	11.50	15.51
	M4	74.64	77.85	65.08	67.96	17.17	20.01	15.94	17.47
	M5	51.72	65.53	43.86	57.94	10.86	14.42	10.00	12.69
	M6	47.01	64.03	44.02	55.11	10.39	14.17	9.86	12.13
	M7	45.42	56.74	40.05	47.10	9.08	12.28	8.25	9.73
	M8	47.78	55.56	41.93	44.14	9.56	11.84	8.72	9.02
T3	**M1**	133.29	141.05	133.58	140.48	27.44	38.08	32.13	37.93
	M2	145.22	155.12	143.88	149.65	38.05	42.92	37.95	40.77
	M3	134.91	153.15	136.45	148.47	30.36	39.67	34.29	39.15
	M4	146.91	156.67	141.86	152.35	38.93	43.23	37.74	41.03
	M5	125.38	145.83	144.59	138.25	29.69	39.92	29.26	36.00
	M6	123.02	142.12	130.96	136.18	24.11	37.67	31.71	35.00
	M7	113.67	139.22	82.94	126.58	27.75	35.45	19.74	29.99
	M8	113.78	138.79	82.42	127.45	28.44	34.84	19.45	31.30
T4	**M1**	134.67	147.43	122.48	133.03	31.92	35.38	31.11	31.82
	M2	142.43	154.76	134.31	147.97	34.18	38.97	35.92	39.84
	M3	127.89	145.30	118.28	132.78	31.72	36.62	30.52	33.41
	M4	140.12	154.49	134.48	148.89	35.03	39.55	36.04	39.90
	M5	124.17	133.13	113.40	113.25	29.80	33.02	27.67	28.09
	M6	136.82	131.10	129.39	105.63	33.11	32.78	31.31	26.34
	M7	75.33	126.37	72.20	97.72	17.83	22.29	16.89	23.54
	M8	72.22	126.08	71.82	100.93	18.39	22.63	16.45	24.08
LSD at 0.05		4.73	3.85	5.00	4.53	1.48	1.16	1.26	1.11

*** 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 leaves fresh and dry weights of *Stevia rebaudiana* L. plants in 2019 and 2020 seasons for two cuts.

Fertilization	Natural ex. Methods app	Leaves fresh weight (g/plant)				Leaves dry weight (g/plant)			
		2019		2020		2019		2020	
		1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut
T1		88.37	89.59	84.23	86.44	23.92	24.24	22.80	23.37
T2		27.53	36.30	24.87	29.37	6.01	7.92	5.44	6.42
T3		83.27	90.84	75.49	89.05	22.09	24.07	20.25	23.72
T4		79.15	82.29	72.55	81.98	18.79	19.57	18.21	20.49
LSD at 0.05		1.17	0.78	1.06	0.64	0.28	0.18	0.29	0.17
	M1	71.80	79.61	67.76	75.00	18.92	20.87	18.28	20.22
	M2	81.82	87.58	78.12	81.77	22.46	23.85	21.92	22.89
	M3	70.66	79.16	64.61	74.38	18.39	20.44	17.41	19.98
	M4	79.56	83.96	76.68	79.31	21.20	22.37	21.24	21.87
	M5	67.80	73.45	62.31	69.78	16.71	18.03	15.33	17.17
	M6	66.78	70.54	58.47	67.84	16.32	17.16	14.29	16.53
	M7	59.54	61.79	53.21	62.92	13.87	14.37	12.43	14.64
	M8	58.68	61.94	53.14	62.69	13.76	14.51	12.50	14.71
LSD at 0.05		1.15	0.95	1.09	0.90	0.28	0.24	0.28	0.23
T1	**M1**	88.53	91.40	87.80	89.24	24.79	25.59	24.58	24.99
	M2	100.34	102.20	95.33	96.38	29.10	29.64	27.65	27.95
	M3	92.80	93.28	85.32	87.79	25.98	26.12	23.89	24.58
	M4	97.28	97.08	94.03	93.15	27.73	27.67	26.80	26.55
	M5	87.65	86.45	82.61	84.99	22.79	22.48	21.48	22.10
	M6	88.38	83.27	81.29	82.74	22.98	21.65	21.14	21.51
	M7	78.62	81.33	74.36	78.08	19.66	20.33	18.59	19.52
	M8	73.38	81.72	73.07	79.12	18.34	20.43	18.27	19.78
T2	**M1**	32.70	42.29	28.73	32.34	6.87	8.88	6.03	6.79
	M2	35.73	47.94	34.61	39.52	8.22	11.03	7.96	9.09
	M3	29.27	39.94	27.12	34.83	6.44	8.79	5.97	7.66
	M4	38.80	44.02	35.07	36.72	8.92	10.12	8.07	8.45
	M5	23.67	34.17	21.87	27.06	5.21	7.52	4.81	5.95
	M6	22.53	31.60	18.87	25.10	4.96	6.95	4.15	5.52
	M7	18.60	25.48	15.80	20.16	3.72	5.10	3.16	4.03
	M8	18.93	24.96	16.93	19.24	3.79	4.99	3.39	3.85
T3	**M1**	84.30	93.80	79.47	91.80	23.60	26.26	22.25	25.70
	M2	97.71	105.27	93.31	97.87	28.69	30.53	27.53	28.87
	M3	82.65	95.20	75.39	92.82	22.04	25.23	20.73	25.53
	M4	89.70	102.60	91.35	96.54	25.12	28.73	26.03	27.13
	M5	83.11	91.36	69.62	87.45	21.19	23.30	17.75	22.30
	M6	81.79	89.43	67.01	85.28	20.45	22.36	16.75	21.32
	M7	72.75	74.71	63.59	80.92	17.46	17.93	15.26	19.42
	M8	74.14	74.34	64.18	79.72	18.16	18.21	15.72	19.53
T4	**M1**	81.66	90.96	75.03	86.61	20.42	22.74	20.26	23.39
	M2	93.48	94.92	89.21	93.28	23.84	24.20	24.53	25.65
	M3	77.92	88.21	70.60	82.10	19.09	21.61	19.06	22.17
	M4	92.47	92.16	86.26	90.85	23.03	22.95	24.07	25.35
	M5	76.78	81.80	75.13	79.61	17.66	18.81	17.28	18.31
	M6	74.41	77.86	66.70	78.23	16.89	17.67	15.14	17.76
	M7	68.20	65.64	59.07	72.50	14.66	14.11	12.70	15.59
	M8	68.27	66.76	58.36	72.68	14.75	14.42	12.61	15.70
LSD at 0.05		2.29	1.91	2.19	1.81	0.57	0.48	0.56	0.47

*** 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.**

seasons. However, distilled water as foliar or drench addition (M_7 & M_8) tended to be the least effective in this regard.

Concerning the response of stevia fresh and dry leaves weights to the interaction between fertilization and natural extracts, application methods followed the same trend as previously detected in leaves area the recommended dose of chemical fertilizer and Azolla aqueous extract at 50% as a drench addition ($T_1 \times M_2$) gave the highest values for the first cut only in both seasons .while in the second cut the highest values were recorded by Azolla aqueous extract combining with 50% NPK +5000 kg h^{-1} ($T_3 \times M_2$) in both seasons. On the other hand, the least fresh and dry weights of leaves values were reported by the interaction between 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, while the second cut had the same trend with the first but with distilled water as a drench addition ($T_2 \times M_8$).

Stem diameter and dry matter percentage:

Referring to the response of stem diameter and dry matter percentage, data presented in Table (12) show that fertilization especially of recommended dose of chemical fertilizer (T_1) had the comparative advantage in the stem diameter in 1st cut as well as 2nd cut in both seasons as registered the highest values. Besides dry matter percentage took the same line with stem diameter in the first cut only as (T_1) gave the highest dry matter percentage followed by 50% NPK + 5000 kg h^{-1} biogas fertilizer (T_3) which had non-significant difference between them in the second cut of both seasons

Both measurements responded positively and significantly to various natural extracts treatment. Herein, yucca aqueous extract as a foliar spray (M_5) registered the largest thickness of the stem diameter for the first cut as well as the second cut in both seasons although the M_6 treatment recorded the highest values in the first cut without significant variance with M_5 while plants treated with Azolla aqueous extract as a drench addition (M_2) recorded the highest dry matter percentage in the cuts which took the same line without significant variation with M_4.

On the other hand, the interaction between recommended dose of chemical fertilizer and yucca aqueous extract as a foliar spray ($T_1 \times M_5$) registered the thickest stem diameter values in the first and second cuts of both seasons while dry matter percentage was recorded the highest values in combination between recommended dose of chemical fertilizer and Azolla aqueous extract as a drench addition ($T_1 \times M_2$) in the first cut in both seasons. In the second cut was recorded by 50% NPK + 5000 kg h^{-1} with Azolla aqueous extract as a drench addition ($T_3 \times M_2$) without significant variance with ($T_1 \times M_4$). As for $T_2 \times M_7$ and $T_2 \times M_8$ (interaction between the recommended dose of biogas fertilizer and distilled water foliar spray as well as drench addition) without significant difference values between them was relatively the least effective in this case.

Essential oil percentage and yield:

Data presented in Table (13) suggested that recommended dose of chemical fertilizer (T_1) registered the richest essential oil percentage and essential oil yield in the first and second cuts of both seasons followed by 50% NPK +5000 kg h^{-1} (T_3) where it has no significant values with (T_1) in the second cut on essential oil percentage. while recommended dose of biogas fertilizer (T_2) recorded the minimum values of the essential oil percentage and yield.

As for natural extracts treatments, data presented in the same Table showed that, garlic aqueous extract, as well as Azolla aqueous extract, gave the highest percentage of the essential oil and yield in the first and second cuts of both seasons without major variations between them. Moreover, M_7 & M_8 (distilled water as a foliar or drench

Table 12. Effect of biogas, chemical fertilizer, and natural extracts and their interaction on stem diameter and dry matter % of *Stevia rebaudiana* L. plants in 2019 and 2020 seasons for two cuts.

Fertilization	Natural ex. Methods app	Stem diameter (cm^2)				Dry matter (%)			
		2019		2020		2019		2020	
		1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut
T1		0.26	0.35	0.28	0.37	26.19	26.50	25.97	26.23
T2		0.18	0.26	0.18	0.28	21.95	23.18	22.79	22.92
T3		0.25	0.30	0.26	0.33	23.57	26.58	24.25	25.94
T4		0.21	0.31	0.24	0.33	24.36	23.17	24.96	25.06
LSD at 0.05		0.01	0.02	0.01	0.02	0.32	0.38	0.25	0.29
	M1	0.21	0.30	0.23	0.31	23.20	25.23	24.64	25.04
	M2	0.23	0.31	0.24	0.34	25.66	26.22	26.29	26.80
	M3	0.21	0.28	0.23	0.32	24.17	25.48	25.20	25.38
	M4	0.25	0.33	0.26	0.37	25.45	26.60	25.88	26.73
	M5	0.26	0.39	0.28	0.40	24.18	25.30	23.56	24.96
	M6	0.27	0.32	0.26	0.37	22.73	25.06	23.85	24.94
	M7	0.19	0.25	0.21	0.27	23.08	22.46	23.35	23.15
	M8	0.20	0.24	0.21	0.26	23.67	22.52	23.18	23.31
LSD at 0.05		0.01	0.02	0.01	0.02	0.52	0.38	0.36	0.29
T1	**M1**	0.23	0.35	0.25	0.34	25.50	26.00	25.70	25.93
	M2	0.25	0.36	0.27	0.37	28.46	27.30	27.94	27.20
	M3	0.21	0.29	0.26	0.33	26.89	26.80	26.16	26.45
	M4	0.27	0.35	0.28	0.41	27.30	27.50	25.62	27.50
	M5	0.35	0.49	0.38	0.47	28.00	27.02	26.80	27.11
	M6	0.33	0.43	0.33	0.44	25.00	26.58	24.56	27.13
	M7	0.23	0.27	0.24	0.30	24.20	25.09	25.60	24.13
	M8	0.24	0.27	0.24	0.28	24.20	25.74	25.40	24.38
T2	**M1**	0.16	0.22	0.17	0.23	23.00	23.90	23.40	23.31
	M2	0.18	0.25	0.17	0.26	24.00	24.70	24.10	25.84
	M3	0.17	0.24	0.16	0.24	22.50	24.00	23.70	23.56
	M4	0.21	0.31	0.21	0.31	23.00	25.70	24.50	25.70
	M5	0.22	0.35	0.23	0.33	21.00	22.00	22.80	21.90
	M6	0.22	0.23	0.19	0.32	22.10	22.13	22.40	22.00
	M7	0.15	0.24	0.16	0.27	20.00	21.67	20.60	20.66
	M8	0.16	0.22	0.17	0.25	20.00	21.32	20.80	20.42
T3	**M1**	0.23	0.33	0.25	0.33	20.59	27.00	24.05	27.00
	M2	0.25	0.32	0.26	0.35	26.20	27.68	26.38	27.24
	M3	0.23	0.30	0.26	0.34	22.50	25.90	25.13	26.37
	M4	0.27	0.33	0.28	0.38	26.50	27.59	26.61	26.93
	M5	0.31	0.35	0.28	0.39	23.71	27.37	20.24	26.04
	M6	0.31	0.26	0.27	0.36	19.61	26.52	24.22	25.70
	M7	0.20	0.24	0.25	0.27	24.43	25.46	23.80	23.69
	M8	0.21	0.25	0.24	0.25	25.00	25.10	23.60	24.57
T4	**M1**	0.24	0.29	0.24	0.34	23.70	24.00	25.40	23.93
	M2	0.23	0.32	0.26	0.36	24.00	25.19	26.75	26.93
	M3	0.22	0.30	0.26	0.35	24.80	25.20	25.80	25.16
	M4	0.25	0.35	0.27	0.38	25.00	25.60	26.80	26.80
	M5	0.18	0.37	0.24	0.39	24.00	24.80	24.40	24.80
	M6	0.21	0.35	0.26	0.36	24.20	25.00	24.20	24.94
	M7	0.19	0.23	0.19	0.23	23.67	17.64	23.40	24.09
	M8	0.19	0.24	0.18	0.24	25.48	17.95	22.90	23.85
LSD at 0.05		0.02	0.05	0.03	0.04	1.04	0.77	0.71	0.59

*** 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 essential oil percentage and yield of *Stevia rebaudiana* L. plants in 2019 and 2020 seasons for two cuts.

Fertilization	Natural ex. Methods app	Essential oil (%)				Essential oil yield l ha^{-1}			
		2019		2020		2019		2020	
		1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut
T1		0.32	0.37	0.32	0.38	8.37	10.24	7.88	10.04
T2		0.16	0.18	0.19	0.21	1.39	2.02	1.60	2.00
T3		0.29	0.36	0.28	0.37	6.42	10.13	6.34	9.77
T4		0.31	0.35	0.31	0.35	6.56	8.59	6.52	8.06
LSD at 0.05		0.01	0.01	0.01	0.01	0.29	0.18	0.12	0.13
	M1	0.24	0.31	0.27	0.33	5.02	7.65	5.66	7.59
	M2	0.30	0.36	0.31	0.38	7.51	9.85	7.41	9.81
	M3	0.29	0.33	0.28	0.33	6.23	8.38	6.07	7.91
	M4	0.31	0.36	0.31	0.38	7.76	10.02	7.45	9.86
	M5	0.28	0.32	0.29	0.32	6.23	7.98	6.03	7.19
	M6	0.28	0.32	0.28	0.31	5.54	7.67	5.65	6.72
	M7	0.22	0.25	0.24	0.28	3.57	5.21	3.34	5.31
	M8	0.22	0.25	0.24	0.28	3.64	5.21	3.09	5.34
LSD at 0.05		0.01	0.01	0.01	0.01	0.25	0.28	0.23	0.21
T1	M1	0.30	0.37	0.31	0.39	7.66	9.79	7.67	10.04
	M2	0.34	0.39	0.34	0.40	10.29	11.56	9.47	11.20
	M3	0.32	0.36	0.32	0.38	8.83	10.10	8.64	10.15
	M4	0.36	0.43	0.35	0.43	10.60	12.70	9.33	12.34
	M5	0.33	0.36	0.33	0.36	10.01	10.74	9.44	10.56
	M6	0.32	0.36	0.32	0.36	8.09	10.29	7.77	9.86
	M7	0.30	0.33	0.31	0.35	5.90	8.34	5.60	8.24
	M8	0.29	0.33	0.29	0.33	5.60	8.42	5.12	7.91
T2	M1	0.13	0.16	0.19	0.21	1.10	1.72	1.60	1.78
	M2	0.15	0.18	0.23	0.24	1.69	2.53	2.30	2.99
	M3	0.18	0.20	0.21	0.21	1.71	2.54	1.71	2.35
	M4	0.19	0.21	0.22	0.24	2.39	3.07	2.57	3.02
	M5	0.17	0.21	0.20	0.20	1.36	2.15	1.44	1.83
	M6	0.17	0.19	0.18	0.19	1.27	1.97	1.30	1.69
	M7	0.12	0.12	0.15	0.17	0.76	1.03	0.90	1.17
	M8	0.13	0.13	0.16	0.18	0.87	1.13	1.01	1.16
T3	M1	0.27	0.38	0.29	0.37	5.34	10.42	6.79	10.20
	M2	0.33	0.41	0.32	0.42	9.13	12.77	8.74	12.43
	M3	0.31	0.38	0.28	0.37	6.70	10.85	6.91	10.33
	M4	0.35	0.42	0.34	0.42	9.72	12.92	9.33	12.51
	M5	0.30	0.35	0.31	0.37	6.31	10.15	6.46	9.50
	M6	0.30	0.36	0.26	0.36	5.15	9.76	5.86	9.16
	M7	0.21	0.28	0.26	0.32	4.26	7.24	3.65	6.91
	M8	0.23	0.28	0.21	0.32	4.78	6.94	2.99	7.13
T4	M1	0.26	0.34	0.29	0.36	5.98	8.66	6.57	8.32
	M2	0.36	0.45	0.35	0.44	8.94	12.53	9.14	12.62
	M3	0.34	0.38	0.32	0.37	7.69	10.02	7.03	8.82
	M4	0.33	0.40	0.33	0.40	8.32	11.39	8.56	11.59
	M5	0.34	0.37	0.34	0.34	7.22	8.88	6.77	6.87
	M6	0.32	0.37	0.34	0.33	7.63	8.66	7.67	6.20
	M7	0.26	0.26	0.26	0.29	3.38	4.22	3.21	4.92
	M8	0.25	0.27	0.27	0.30	3.31	4.35	3.24	5.14
LSD at 0.05		0.02	0.02	0.02	0.02	0.49	0.55	0.47	0.42

*** 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.**

Addition) tended to be the least effective in this respect.

Furthermore, the interaction between the recommended dose of chemical fertilizer and garlic aqueous extract as a drench addition ($T_1 \times M_4$) treatment shares importance with 50% NPK+3000 kg h^{-1} combining with Azolla aqueous extract as a drench addition ($T_4 \times M_2$) which recorded the highest essential oil percentage without non-significant variation between them. As for essential oil yield, the results in the same Table showed that both $T_1 \times M_4$ and $T_1 \times M_2$ treatments registered the highest values in the first cut of both seasons, while in the second cut ($T_3 \times M_4$) and ($T_4 \times M_2$) recorded the highest essential oil yield without non-significant variation between them.

On the other hand, the minimum values were recorded by interaction between the recommended dose of biogas fertilizer and distilled water foliar spray as well as drench addition ($T_2 \times M_7$ and $T_2 \times M_8$) without significant difference values between them.

Essential oil GC-mass analysis:

Data presented in Table (14) and Figs. (1-3) showed that the total compounds representing 90.44–94.37% of the total detected constituents with different treatments. The unknown compounds representing 2.12–9.56% of the total detected constituents in the two seasons. The main constituents of *Stevia rebaudiana* essential oil detected by GC-MS were β farnesene (19.46, 5.24, 42.5%), cardinol (19.54, 1.66, 12.73%), á-farnesen (1.46, 13.64, 13.64%), and caryophyllene (2.21, 13.71, 0.86%). The highest β farnesene was achieved with the recommended dose of chemical fertilizer + garlic aqueous extract as a drench addition treatment ($T_1 \times M_4$). The same trend was detected á-farnesene while the reverse was true with Caryophyllene which presented 13.71% with treatment ($T_4 \times M_2$). The results agreed with (Gasmalla *et al*., 2017) who found that the highest percentage of the compound β farnesene was concentrated in the stem, which confirm our results that all the aerial parts especially stem and leaves contain essential oil.

In addition to the caryophyllene compound, was found in ratio of treatment ($T_4 \times M_2$), which is the half dose of chemical fertilizer and organic fertilizer (biogas) treatments with garlic extract, and it is considered one of the most famous oil compounds and the most economical.

DISCUSSION

Growth parameters, broadly speaking, refers to the irreversible increase in the shape, size, and weight of the plant. A higher growth rate means more accumulation of fresh and/or dry weight by the plant resulting in higher yield. This growth and yield relationship may be affected by various factors like climatic, edaphic, and topographic factors. Among them, one of the most important factors is the maintenance of appropriate nutrient status in the soil. The organic manures, critical resources, are said to contribute to the organic matter and nutrient status of the soil and their favorable effects mediated through improved nutrients. All plant parameters like plant height, number of branches fresh and dry weight of shoots and leaves, and leaf area in the early growth period of organic manure cultivation are relative to the production of chemical fertilizers. The increase in the growth rate in general in the first cut is due to chemical fertilizer as the presence of soft elements ready for the plant in a suitable form for absorption. However, in the second cut, a process of decomposition of the biogas organic fertilizer occurred that improves physical, chemical, and biological properties of the soil; that is, increasing soil organic matter, cation exchange capacity, water holding capacity and availability of mineral nutrients and, thus, in turn, increase growth rate (Al-Fraihat *et al*., 2011)**.** These results are in agreement with (Verma *et al*., 2020) on Stevia plant, they reported that the growth parameters, *i.e.*, plant height (cm), number of leaves per plant, and the number of branches per plant, were significantly higher with

Table 14. Chemical composition of the essential oils of *Stevia rebaudiana* L. plant using GC-MS for the first cut in the second season.

Ref.	Rt (min)	Name of compound	T_2M_7*	T_4M_2**	T_1M_4***
1	3.95	α-Pinene	1.08	-	3.57
2	9.58	(-)-á-Bourbonene	-	0.28	-
3	9.73	beta.-Elemene	0.57	3.06	-
4	10.05	Ethyl 9,12-hexadecadienoate	0.57	-	-
5	10.27	Caryophyllene	2.21	13.71	0.86
6	10.56	cis-à-Bergamotene	0.52	3.17	-
7	10.96	(E)-á-Famesene	1.46	13.64	13.64
8	11.47	á-copaene	0.98	-	1
9	11.49	Germacrene D	-	8.14	-
10	11.79	Elemene isomer	1.56	8.95	-
11	11.9	b Farnesene	19.46	5.24	42.5
12	12.15	ç-Muurolene	0.36	1.19	-
13	13.3	d-Cadinene	-	2.38	-
14	12.7	Naphthalene 1 2-dihydro-1 5 8-trimethyl	0.27	-	-
15	13.09	Nerolidol	1.15	5.5	-
16	13.39	Spathulenol	1.55	5.08	-
17	13.48	Caryophyllene oxide	0.8	4.17	-
18	13.65	Globulol	-	0.25	-
19	13.74	Á-Longipinene	-	0.44	-
20	13.86	Ledol	-	0.29	-
21	13.97	Humulene epoxide	0.28	1.64	-
22	14.28	Cubenol	-	0.27	-
23	14.4	Elemol	-	0.54	-
24	14.57	.Tau.-cadinol	0.77	2.92	-
25	14.68	Alloaromadendrene	-	0.16	-
26	14.83	À-Cadinol	0.74	3.05	-
27	15.13	Caryophyllene epoxide	0.88	1.26	-
28	15.49	Eudesma-4(14),7(11)-diene	0.42	0.36	-
29	15.69	A-Terpineol	2.22	1.66	-
30	16	Isoaromadendrene epoxide	1.88	-	1.88
31	16.48	11,13-Dihydroxy-tetradec-5-ynoicacid, methyl ester	0.54	-	-
32	16.74	Ledene oxide	-	0.3	-
33	17.4	Alloaromadendrenoxid	0.48	-	-
34	17.67	Nd	0.4	-	-
35	18.07	Aromadendrene oxide	0.24	-	-
36	18.17	Nd	0.26	0.42	-
37	19.54	(E)-jasmone	0.82	0.55	0.65
38	20.27	Palmitic acid	3.18	-	2.24
39	20.61	1H-Naphtho[2,1-b]pyran, 3-ethenyldodecah	0.86	5.95	-
40	22.06	Nd	-	0.67	-
41	22.21	Linoleic acid ethyl ester	0.55	-	0.51
42	22.31	Nd	0.98	0.36	1.04
43	22.48	Nd	3.63	0.67	8.13
44	22.72	Nd	0.19	-	-
45	22.99	Cardinol	19.54	1.66	12.73
46	24.56	Z-(13,14-Epoxy)tetradec-11-en-1-olacetate	1.24	-	-
47	25.21	1,2-15,16-diepoxyhexadecane	1.84	0.37	0.81
48	25.53	Farnesol	0.53	-	1.97
49	26.61	Nd	0.18	-	-
50	26.67	Heptacosane	0.45	-	-
51	27.01	T-Cadinol	7.71	0.78	3.66
52	27.37	4,8,13-Duvatriene-1,3-diol	0.92	-	-
53	27.97	2-hydroxy-3-[(9e)-9-octadec enoyloxy]propyl	0.89	-	-
54	27.63	A-Cadinol	8.2	0.91	4.42
55	30.06	9-Octadecenoic acid (Z)-methyl ester	6.65	-	-
Total identified			94.37	97.87	90.44
Total			100.01	99.99	100

***T_2M_7 recommended dose of biogas fertilizer * distilled water as a foliar spray.**
****T_4*M_2 50%NPK+3000 Kg biogas fertilizer * Azolla aqueous extract as drench addition.**
*****T_1M_4 recommended dose of chemical fertilizer * garlic aqueous extract as drench addition.**

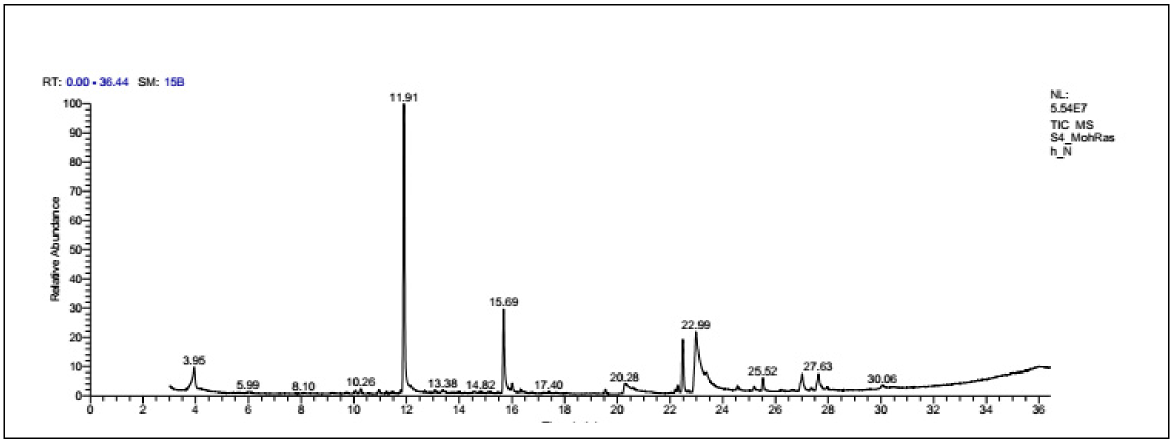

Fig. 1. Effect of recommended dose of chemical fertilizer with garlic extract as drench addition on the essential oil constituents using GC/Mass analysis.

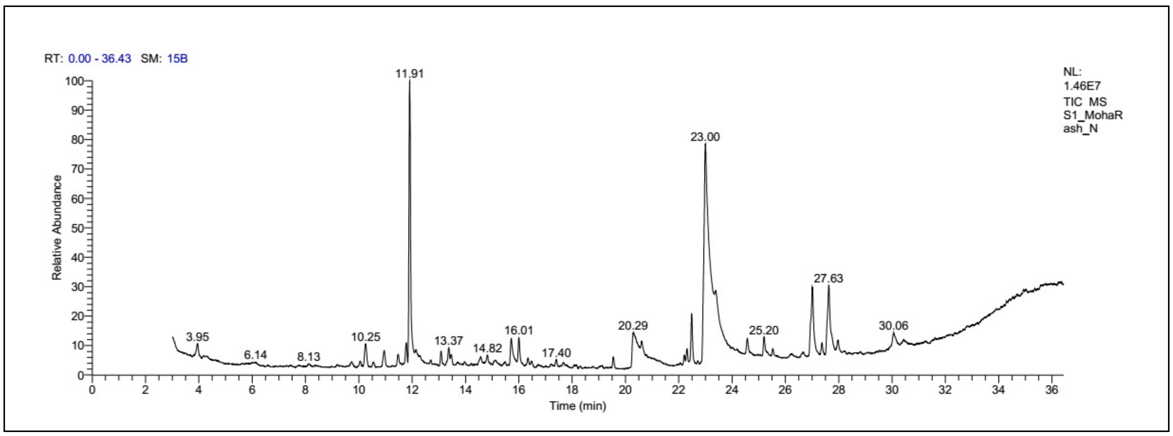

Fig. 2. Effect of recommended dose of biogas fertilizer with distilled water as foliar spray on the essential oil constituents using GC/Mass analysis.

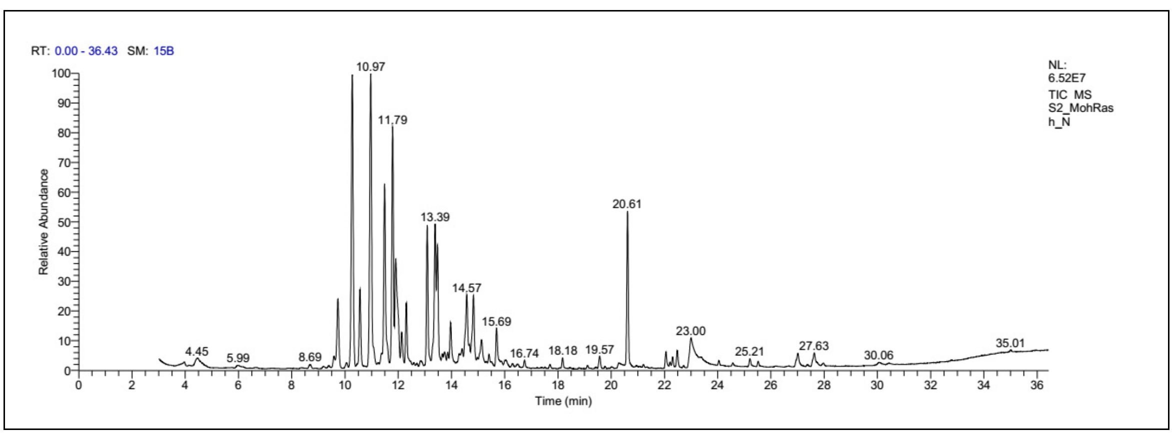

Fig. 3. Effect of 50% recommended dose of chemical fertilizer with Azolla aqueous extract as drench addition on the essential oil constituents using GC/Mass analysis.

nitrogen at 300 kg/ha, which has no significant differences to 200 kg/ha. (Martínez *et al*., 2018) concluded that vermicompost at 4 g N plant-1 increased leaves and buds per plant of stevia from 3 buds and 12 g to 9 buds and 19 g leaves, respectively, compared to chicken manure treatment at the same dosage. (Lam, 2011) on tea plant treated with slurry, tea improves in quality and the yields increase by 11%. This is a net saving of around €148 per hectare per harvest.

Subsequently, increasing plant height refers to yucca aqueous extract, which works to break the surface tension of the leaf because it contains steroidal saponins, so it facilitates gas exchange, which enhances the plant's ability to perform photosynthesis and water and food absorption The results are in harmony with (Wulff *et al*., 2012) who reported that the application of aqueous saponin-rich extracts of *Digitalis purpurea* stimulated the formation of adventitious roots on tomato cuttings while application of the pure saponin, digitoxin, had the opposite effect, indicating toxicity to tomato plants.

On the other hand, the increase in number of branches, number of leaves, leaf area, shoots dry weight, and essential oil percentage may be due to the biochemical function of vitamins and amino acids in the garlic as well as Azolla aqueous extract, which has enhanced the role of metabolic processes and the levels of endogenic hormones, i.e. IAA and GA_3 (Elzaawely *et al*., 2018). Garlic extract led to cell proliferation, cell enlargement, and cell division, which has contributed to an increase in the number of leaves. These effects can also be due to the influence of garlic extract on growing levels of endogenous hormones in treated plants, (Khedr and Farid, 2000). These observations were consistent with (AbdelKader *et al*., 2014), (Ziedan and Eisa, 2016) on *Anethum graveolens* (Massoud *et al*., 2017) on *Majorana hortensis* L. plant. (El-Said and Ali, 2013) on *Cumin*. Likewise, (Hanafy *et al*., 2012) reported that the highest values of plant height and number of branches contents in schefflera treated with garlic aqueous extract as drench addition.

This increase in the leaf's dry weight may be due to an increment in number of leaves and leaf area. Also, this may be due to Azolla extract which contains a lot of amino acids vitamin and some of the growth regulators which enhance plant growth, number of leaves which led to increasing leaves fresh, and dry weight. These results were in harmony with (Rabie *et al*., 2020) and (Kawtar *et al*., 2017) on *Matricaria chamomilla* L. who showed that application of organic fertilizer with Azolla extracts achieved the highest growth parameters in both seasons.

On the other hand, essential oil chemical analysis agreed with (Marculescu *et al*., 2002) as they revealed that the use of organic fertilizers plays an essential role in the plant's development, in the biosynthesis of the organic substances at all levels, also it can be noted that when using manure the amount of active principle (essential oil) is high in chrysanthemum plant. The effect of different treatments on essential oil yield and constituents may be due to garlic extract chemical composition which enhances their effect on enzyme activity and metabolism of essential oil production (El-Said and Ali, 2013).

CONCLUSION

Organic fertilizer, especially biogas, is characterized by an increase in its nitrogen content, which enhances the vegetative characteristics of the plant, but it needs a period for its analysis, which appeared clearly in the second cut of both seasons. The results showed that the use of half (50%) of the recommended dose of chemical fertilizer with the equivalent of the recommended nitrogen content and replaced it with biogas fertilizer led to reducing the problem of the use of chemical fertilizers on the human health and economic damages, in addition to the use of both extracts of Azolla and garlic as a drench additive with biogas as organic fertilizer and half the dose of chemical fertilizer.

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التأثيرالتعاونى التحفيزى لسماد البيوجاز والمستخلصات الطبيعية كالثوم والازولا واليوكا على النمو الخضري ومحصول الزيت العطرى على نبات الاستيفيا تحت ظروف جنوب سيناء

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يعتبر نبات الاستيفيا من أفضل المصادر الطبيعية البديلة للسكريات الاصطناعية مثل الأسبارتام والسكرين لمرضى السكر. يوفر هذ النوع من السكر العديد من الفوائد الطبية ، بما في ذلك ضبط مستوى ضغط الدم ، وضبط مستوى السكر ، ومضادات الالتهاب ، ومضادات الأورام ، ومضادات الأكسدة، وما إلى ذلك. أجريت تجربتان ميدانيتان في موسمي ٢٠١٩/٢٠١٨ و ٢٠٢٠/٢٠١٩ بالمزرعة التجريبية برأس سدر بجنوب سيناء والتابعة لمركز بحوث الصحراء بالقاهرة بالتعاون مع قسم البساتين بكلية الزراعة. جامعة بنها ، لدراسة تأثير سماد البيوجاز والمستخلصات الطبيعية على الصفات النباتية ونسبة ومحصول الزيت العطري لنبات الاستيفيا. أظهرت النتائج أن سماد البيوجاز مع المستخلصات الطبيعية المختلفة كان لهما تأثير كبير على خصائص النمو ومحتوى الزيت العطري للاستيفيا. تم الحصول على أعلى القيم في معظم المعاملات في الحشة الأولي من تفاعل الجرعة الموصى بها من السماد الكيميائي (T_1) مع مستخلصات الثوم كاضافة

ارضية ومستخلص اليوكا رشاً ومستخلص الازولا إضافة ارضية (M_4 أو M_5 أو M_2). من ناحية أخرى، أخذت الحشة الثانية نفس الاتجاه مع الحشة الأولي ولكن مع نصف الجرعة الموصى بها من السماد الكيماوى +٥٠٠٠ كجم من سماد البيوجاز (T_3) مثل ($T_3 \times M_4$) أو ($T_3 \times M_2$) حيث سجلت الاضافة الارضية لكل من مستخلصى الثوم والازولا أعلى قيم المعاملات المذكورة أعلاه في معظم الحالات. بشكل قاطع فإن استخدام نصف الجرعة الموصى بها من السماد الكيماوي واستبداله بأسمدة البيوجاز بما يعادل محتوى النيتروجين الموصى به أدى إلى التقليل من مشكلة استخدام الأسمدة الكيماوية على الصحة والأضرار الاقتصادية، مع التعزيز باضافة استخدام مستخلصي الأزولا والثوم ادى الى الحصول على افضل النتائج فى اغلب الصفات.