Response of Stevia to Salicylic Acid, Salinity Remediator and Plant Density under Soil as Affected by Salinity

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ABSTRACT: A field experiment was conducted at the Experimental Research Farm of Faculty of Agriclture, Saba Basha, Alexandria Governorate, Egypt in years 2022 and 2023, to examine how *stevia rebaudiana* respond to application of some salicylic acid (SA), salinity remediator (SR=Cetro_Cal) and plant density under saline soil condition. In growing season, this factorial experiment was set up in a split plot design with three replications. The four salicylic acid (SA), salinity remediator (SR=Cetro_Cal) treatments (control= untreated, salinity remediator (Cetro-Cal) at the rate of 4 L/fed, foliar application of salicylic acid (SA) at the rate of 200 mg/l and application of SR (4 L/fed) + SA (200 mg/l) were allocated in the main plots and the three-plant spaces viz., 40 cm, 26.7 cm and 20 cm with densities (20000, 30000 and 40000 plants/fed), respectively were distributed at random within the subplots.The results showed that stevia plants reponsed to the different salicylic acid (SA), salinity remediator (SR=Cetro_Cal) treatments, plant densities and their interaction under study conditions. Ultimately, the findings suggested that using Citric -Cal, and SA under saline conditions will boost stevia growth , and yield at a planting density of 40000 plants /fed (20 cm apart between plant).

Keywords: *stevia, plant density, salicylic acid, salinity, remediator, growth, yield.*

INTRODUCTION

Stevia (*Stevia rebaudiana* Bertoni) is a perennial herb commonly known as sweet weed, sweetleaf, sweet herbs, sugar leaf and honey leaf, belongs to the Asteraceae (Compositae) family native to Brazil and Paraguay, where it can be a new promising crop **(Tavarini and Angelini, 2013; Halim *et al*., 2016).** It is well known that the stevia leaf contains sweet-tasting diterpenoid steviol gly-cosides that are 300 times sweeter than sucrose. Stevioside and Rebaudioside are dominant components amongst the known steviol glycosides (**Kumar *et al*., 2013**).

Salinity remediators (SR) contains organic carboxylic acids, calcium (Ca), amino acids, nitrogen (N), and magnesium. The salinity processor works by breaking the bonding of the sodium (Na^+) element to the soil and replacing it with calcium (Ca), allowing Na to be washed to the lower layers of the soil, as well as releasing the element chlorine (Cl^-), which becomes free and readily washed and neutralized. Low-molecular-weight osmolytes, such as glycine betaine, proline, and other amino acids, organic acids, and polyols, are essential for cellular function maintenance under drought or salt stress (**Farooq *et al*., 2009**). Organic and Ca-based additions have the potential to increase agricultural production in salty soils (**Niamat *et al*., 2019**). In comparison to the control treatment, the administration of 3 percent Ca-FCM resulted in the greatest reduction in Na+/K+ ratio (**Naveed *et al*., 2020**).

Salicylic acid (SA), one of the plant growth regulators. It plays an important signaling role in the activation of numerous defense responses of plants (**Yu *et al*., 2006**). SA (or 2-hydroxybenzoic acid), is known as an endogenous phytohormone with phenolic nature that has a remarkable role in the growth and development of plants (**Belt *et al*., 2017**).SA enhances tolerance toward most kinds of abiotic stress due to an enhanced antioxidant capacity **(Horváth *et al*., 2007**). Foliar spraying with salicylic acid at 100 mg/l concentration gave the highest significant values for growth characters of stevia under salinity condition **(El-Housini *et al*., 2014).**

Plant density is one of the most important cultural practices determining herbage yield, as well as other important agronomic attributes of this crop (**Taleie *et al*., 2012**). Plant density and harvesting time are factors that affect biomass and sweetening compounds and yield in this species (**Gomes *et al*., 2018; Francisco *et al*., 2018**). Narrow row spacing may also increase the competitive ability of a crop. Decreasing row spacing may also limit the period that weeds can compete with crops. The competitive advantage of narrow rows may also contribute to increased crop yield **(Kumar *et al*., 2012).** The growth, yield and quality of Stevia leaf were significantly affected by plant density and cutting time (**Serfaty *et al*., 2013**). Higher yields in high densities are a function of the greater number of plants per area, which compensates for the great biomass production per plant where narrow plant spacing's increased both plant height and yield (**Kumar *et al*., 201**4). The highest planting density promoted an average leaf yield of 1053.67 kg/ha. This value is close to the maximum reported by **Pal *et al.* (2013, 2015).** Because the plants showed the best performance in the second year due to good adaptation to environmental condition, better yields were obtained at the plots with narrow plants spacing in both harvests done in spring and summer season. Leaf yields were higher during flowering. As the plant continues to grow from vegetative to generative stage. According to the result obtained from the research, it has been indicated that stevia plants can be successfully grown. Also, there were morphological differences between the seedling from seeds directly. Further research is needed to select the best seedlings and to determine the yield and quality characteristics (**Tansı *et al*., 2017**). Also, the growing and flowering of Stevia were affected by day length, temperature, soil moisture and wind (**Ramesh *et al*., 2006**).

Therefore, field experiment was conducted to evaluate the influence of some salicylic acid (SA), salinity remediator (SR=Cetro_Cal) treatments, planting densities and their interaction on the growth, yield and protein of stevia under salty soil.

MATERIALS AND METHODS

Experimental site

The field experiment was conducted at the Experimental Research Farm - Faculty of Agriculture, Saba Basha, Abees Region, Alexandria Governorate, Egypt, to investigate the response of stevia to salicylic Acid (SA), salinity remediator (SR= Cetro- Cal) under the different plant densities under salinity conditions from September 2022 to September 2023.

Representative soil samples from depth (0 – 60 cm) were taken from the experimental soil before starting the experimental work. Some soil properties were determined according to the method described by (**Chapman and Pratt, 1961**); **Sparks *et al.* (2020)** and are presented in **Table (1).**

Weather data

The monthly average of the meteorological data noted during the growing period at Alexandria location (Altitude: 32 m, Latitude: 31.36 °N Longitude: 29.95 °E, Egypt according to the FAO database using CLIMWAT 8 (**Muñoz and Grieser, 2006**) are presented in **Table (2)**. According to **Table (2)**, climate conditions and water management may hasten salinization. Evapotranspiration is critical in the pedogenesis of saline and sodic soils in arid lands. According to **(Wanjogu *et al*., 2001),** most arid land get less than 200 mm of rainfall/year, results in salinization.

Table 1. Some soil properties of the experimental sites

Soil Properties	**Values**
Mechanical analysis	
Sand	16.00
Silt	39.70
Clay	44.30
Soil texture	Clay loam
p^H (1:1)	8.20
Chemical properties	
EC (1:1) dS/m	3.60
Soluble cations (1:2) (cmol/kg soil)	
K^+	1.55
Ca^{++}	14.17
Mg^{++}	10.34
Na^+	14.55
Soluble anions (1:2) (cmol/kg soil)	
$CO^-_3 + HCO^-_3$	2.90
CL^-	21.00
SO^-_4	16.70
Calcium carbonate (%)	6.20
Total N (%)	1.20
P (mg/kg)	3.40
Organic matter	1.40

Table (2). Average of minimum, maximum temperature (cº), rainfall (mm) and relative humidity (%) at Alexandria, Egypt.

Month	Temperature (cº)			Rainfall (mm)	Relative humidity (RH%)	Wind (km/day)	Sun (hours)
	Mini.	Maxi.	Average				
January	10.6	18.3	14.5	48.0	82.0	259.0	6.1
February	11.1	18.9	15.0	23.0	80.0	268.0	6.9
March	12.8	21.1	17.0	10.0	80.0	277.0	7.9
April	15.0	23.3	19.2	3.0	83.0	251.0	9.1
May	17.8	26.1	22.0	3.0	870	242.0	9.6
June	20.5	28.3	24.4	3.0	80.0	242.0	10.6
July	22.8	29.4	26.1	3.0	89.0	259.0	10.6
August	20.0	30.5	25.3	3.0	88.0	233.0	10.6
September	22.8	30.0	26.4	3.0	83.0	216.0	9.8
October	20.0	28.3	24.2	5.0	82.0	190.0	8.6
November	16.7	25.0	20.9	33.0	82.0	199.0	7.3
December	12.8	20.5	16.7	56.0	82.0	242.0	5.8
Average	16.9	25.0	20.9	16.1	148.4	239.8	8.6

Experimental detail

Nursery of stevia crop was raised during June 2022 in sand beds through seed. Three-month-old seedlings were transplanted in the field on September 22, 2022.

The experiment consisted of four biostimulant treatments i.e., (control= untreated, salinity remediator (SR = Cetro- Cal) at the rate of 4 L/fed, foliar application of salicylic acid (SA) at the rate of 200 mg/l, and application of SR (4 L/fed) + SA (200 mg/L) and the three-plant density viz., (20000, 30000 and 40000 plants/fed). Each treatment was replicated three times in a split plot design, four biostimulant treatments allocated in main plots while plant density distributed at random in subplots.

The application of SR (Cetro – Cal) at the rate of 4 L/fed and SA at the rate of 200 mg/L were applied four times per growing season starting from March until September 2023 at the same time, the control was sprayed with tap water.

Stevia crop undergoes dormancy during winter months and regrowth of crop starts with rise in temperature during March. Commercial SR called Cetro- Cal contains (5 % organic acid i.e., Citric acid + Amino acid, 8% nitrogen and 11% Ca, while SA properties are presented in Table **3** and were brought from Oasis Company (Egypt) Alexandria Desert Road, whose commercial name is SA Anti-Free.

Table (3). Properties of Salicylic acid (SA) compound.

Chemical		Physicochemical			
Propertie s	Salicylic acid (%)	Appearance	Color	Solubility	pH
Values	99%	Solid powder	Off-white	Totally water - soluble	4-8

Stevia seeds were obtained from Sabhia Station, Sugar Crops Research Institute, Agricultural Research Centre (ARC), Egypt.

The experimental unit area was 10.5 m^2 included 6 ridges, each of which 50 cm width and 3.5 meters long. The distance between plants was applied according to plant densities/fed as following;

Feddan Area(m^2)	Ridge width (cm)	Plant Spacing (cm)	Plant density (plants/fed)
4000	50	40.0	20000
4000	50	26.7	30000
4000	50	20.0	40000

before planting, pre-emergence herbicide (Gardo, 96%; S-metolachlor) was applied at a rate of 125 cm3/fed. Over time, weeds were also manually controlled by hand hoeing. Several farming methods were used to grow stevia in compliance with information made by the Sugar Crops Research Institute, A.R.C..

A common fertilizer dose of NPK/fed at the rate of 50:24:24 kg was applied to supplement the nutritional requirements of stevia crop. In 2022, nitrogen was added in two equal split doses via urea (N = 46%). The first dose was applied at transplantation, and the second was administered two months later. In 2023, however, half of the nitrogen quantity and complete P and K treatments were applied in April, and the remaining half of the nitrogen quantity was applied in June. As suggested by the Sugar Crops Research Institute, A.R.C., other agricultural techniques were employed to cultivate stevia plants.

Young seedlings were transplanted into the field on September 22 2022. Because the new plants have not grown sufficiently. The months of the first year were adopted as the establishment period therefore, the data were excluded. The next year 2023, new shots plants were carefully managed, and the data were collected from the three times, 50 days intervals between cuts, respectively were performed and necessary.

Studied Characteristics

Growth and yield characteristics were recorded three times (cuts) as following,

Plant height (cm), total chlorophyll (SPAD unit), number of branches/plant, dry matter accumulation (gr/plant), total fresh leaves yields/season (ton/fed), total fresh stems yields/season (ton/fed), biological fresh yields/season (ton/fed), total dry leaves yields/season (ton/fed), total dry stems yields/season (ton/fed), biological dry yields/season (ton/fed) and leaves protein content (%) .

The samples were recorded by selecting 10 plants at random from each subplot during the three cuts to study the growth characters.

The yield characteristics were recorded from the two inner ridges which harvest and weighted then separated into (leaves and stems) then transferred to feddan=4200 m^2 during the three cuts (harvest) then summed to get yield in season.

Statistical Analysis

Data obtained was exposed to the proper method of statistical analysis of variance as described by **Gomez and Gomez (1984).** The treatments means were compared using the least significant differences test (LSD) at the 5% level of probability. All the statistics analysis using **CoStat 6.311 (2005)** computer software package.

RESULTS AND DISCUSSION

The results presented in **Tables (4, 5, and 6)** showed the effect of salicylic acid (SA), salinity remediator (SR=Cetro_Cal) application, planting density and their interaction on plant height (cm), total chlorophyll (SPAD unit), number of branches/plant, dry matter accumulation (gr/plant) and Total fresh Leaves, stems and biological yields/season (ton/fed) of stevia plants as affected by salicylic acid (SA), salinity remediator (SR=Cetro_Cal), planting density and their interaction at the three cuttings (cutting) during 2023 seasons.

Concerning the effect of application of salicylic acid (SA), salinity remediator (SR=Cetro_Cal) on growth characters i.e., plant height (cm), total leaf chlorophyll (SPAD unit), number of branches/plant, dry matter accumulation (gr/plant) of stevia at the three cuttings. The results in **Tables (4 and 5)** revealed that the tallest plant height, the highest values of plant height (cm), total chlorophyll (SPAD unit), number of branches/plant, total dry matter accumulation (gr/plant) and Total fresh Leaves, stems and biological yields/season (ton/fed) of stevia were recorded with salinity remediator (Cetro- Cal) at the rate of 4 L/fed combined with salicylic acid (SA) at the rate of 200 mg/l followed by application of SA then salinity remediator, respectively with no significant difference among these treatments while the control treatments= untreated treatments gave the lowest values of these traits in all the cuttings (cuts). An increase of these traits due to the vital role of SA and Cetro- Cal for growth of plants such as stevia plants under abiotic stress as soil salinity. In this investigation, the maximum growth and production of stevia were seen in this inquiry when SA and SR were applied either separately or combined. Due to an increased antioxidant capacity, SA increases tolerance to most abiotic stresses. A natural signal molecule called SA, (2-hydroxy benzoic acid), may contribute to how plants react to diverse conditions (**Horváth *et al*., 2007**). Various plant species have been demonstrated to be protected against abiotic stress factors like salinity by spray with SA solution (**Rajjou *et al*., 2006**).In the other side, in our study application of salinity remediator (SR= Cetro- Cal) increased growth and yield of stevia may be due to structure of these materials where it contains amino acids, Cao, and organic acid, in this trend agrosol fertilizer (salinity remediator) increased growth yield, improved quality of produce, increased resistance to dry and wet stress, decreased water requirement, healthier and more vigorous plant, stronger and heavily branched root system, improved use of available fertilizer, and higher levels of photosynthetic activity (**Gomaa *et al.*, 2021)**.

In terms of response of stevia to planting density, the results in **Tables (4 and 5)** indicated that plant population density (20000 plants/fed) recorded the highest values of plant height (cm), total chlorophyll (SPAD unit), number of branches/plant, and dry matter accumulation (gr/plant) at all cuttings (cuttings) followed by planting stevia plant with 30000 plants/fed as compared with plant density (40000 plants/fed) which gave the lowest values of all the traits during the three harvesting times (cuttings) in 2023 seasons.

Regarding the interaction effect between the two factors as shown in **Tables 4 and 5**. The results showed that sowing stevia plants with 20000 plants/fed with application of SA + Citro- Cal, or with SA or Cetro- Cal increased plant height (cm), total chlorophyll (SPAD unit), number of branches/plant, and dry matter accumulation (gr/plant) in each cutting during the growing season, 2023.

Table (4). Plant height (cm) and total chlorophyll content (SPAD) of stevia as affected by SR, SA, planting density and their interactions in growing season.

Treatments	Plant height (cm)											
	B) Plant density/fed											
	20000	30000	40000	Average	20000	30000	40000	Average	20000	30000	40000	Average
A)Salicylic acid (SA), salinity remediator (SR=Cetro_Cal)	First cut				Second cut				Third cut			
Control= untreated	30.33	31.00	35.67	32.33	31.67	32.50	37.83	34.00	32.83	33.00	40.17	35.33
Salinity remediator (SR)	35.67	42.67	44.00	40.78	37.00	44.00	45.50	42.17	38.17	45.00	46.50	43.22
Salicylic acid (SA)	36.33	40.67	46.00	41.00	37.67	41.67	48.83	42.72	38.83	43.17	51.17	44.39
SR + SA	35.33	41.33	49.67	42.11	37.50	42.83	52.50	44.28	38.17	43.83	54.50	45.50
Average	34.42	38.92	43.84		35.96	40.25	46.17		37.00	41.25	48.09	
LSD at 0.05 for A		2.21				2.44				2.70		
LSD at 0.05 for B		1.74				1.69				1.65		
LSD at 0.05 for A x B		3.49				3.39				3.29		
	Total Chlorophyll (SPAD unit)											
	B) Plant density/fed											
	20000	30000	40000	Average	20000	30000	40000	Average	20000	30000	40000	Average
A)Salicylic acid (SA), salinity remediator (SR=Cetro_Cal)	First cut				Second cut				Third cut			
Control= untreated	36.02	33.05	32.70	33.92	38.33	34.37	32.69	35.13	40.33	36.00	33.00	36.44
Salinity remediator (SR)	45.70	43.70	37.70	42.37	47.06	44.69	38.73	43.49	47.00	45.33	38.67	43.67
Salicylic acid (SA)	46.37	42.03	37.75	42.05	48.04	42.71	38.71	43.15	48.67	43.00	38.50	43.39
SR + SA	50.17	43.38	37.70	43.75	50.00	44.39	38.70	44.36	51.33	49.01	49.00	49.78
Average	44.57	40.54	36.46		45.86	41.54	37.21		46.83	43.34	39.79	
LSD at 0.05 for A		1.59				1.22				1.60		
LSD at 0.05 for B		1.60				1.47				0.68		
LSD at 0.05 for A x B		3.20				2.95				1.37		

SR= Citro- Cal, SA= salicylic acid

Table (5). Number of branches/plant and dry matter (gr/plant) of stevia as affected by SR, SA, planting density and their interactions in growing season.

Treatments	Number of branches/plant											
	A) Plant density/fed											
	20000	30000	40000	Average	20000	30000	40000	Average	20000	30000	40000	Average
A)Salicylic acid (SA), salinity remediator (SR=Cetro_Cal)	First cut				Second cut				Third cut			
Control= untreated	9.48	8.76	8.67	8.97	10.67	10.43	10.48	10.53	12.00	11.00	12.10	11.70
Salinity remediator (SR)	12.57	11.86	9.86	11.43	14.57	13.52	11.86	13.32	16.57	15.52	11.33	14.47
Salicylic acid (SA)	13.14	11.43	10.33	11.63	15.14	13.43	12.33	13.63	15.67	15.10	13.57	14.78
SR + SA	13.67	11.81	10.33	11.94	13.33	13.81	12.33	13.16	15.00	15.81	14.00	14.94
Average	12.22	10.97	9.80		13.43	12.80	11.75		14.81	14.36	12.75	
LSD at 0.05 for A		0.74				1.21				1.27		
LSD at 0.05 for B		0.50				0.60				0.78		
LSD at 0.05 for A x B		0.99				1.20				1.55		
	Total Dry matter accumulation (g/plant)											
A)Salicylic acid (SA), salinity remediator (SR=Cetro_Cal)	First cut				Second cut				Third cut			
	A) Plant density/fed											
	20000	30000	40000	Average	20000	30000	40000	Average	20000	30000	40000	Average
Control= untreated	28.81	24.23	23.52	25.52	29.87	26.8	25.93	27.53	31.61	27.35	26.5	28.49
Salinity remediator (SR)	34.17	32.66	28.03	31.62	36.82	35	30.44	34.09	37.32	35.89	31.61	34.94
Salicylic acid (SA)	35.75	31.63	28.17	31.85	38.33	34.24	30.83	34.47	39.03	34.83	31.46	35.11
SR + SA	38.46	32.14	27.47	32.69	41.37	34.75	30.17	35.43	41.98	35.26	30.95	36.06
Average	34.30	30.17	26.80		36.60	32.70	29.34		37.49	33.33	30.13	
LSD at 0.05 for A		1.43				1.56				1.33		
LSD at 0.05 for B		1.26				1.31				1.25		
LSD at 0.05 for A x B		2.53				2.61				2.50		

SR= Citro- Cal, SA= salicylic acid

The results in **Table 6** revealed that total fresh leaves, stems and biological yields/season (ton/fed) of stevia increased with the highest plant population (40000 plants/fed). The results indicate that a plant population density of up to 40,000 plants/fed, along with the other studied characteristics of stevia, represents the ideal plant population (plant spacing) in feddan. In contrast, a wider spacing resulted in the lowest number of plants/fed, as well as the lowest growth and yield.

Concerning the interaction effect between the two factors as shown in **Table 6**, showed that sowing stevia plants with 40000 plants/fed with application with SA + SR, or with SA or SR increased fresh and dry yields (leaves, stems and biological) in each cutting during the growing season, 2023.

In the current investigation, the wider spacing with a plant population of 20,000 plants/fed resulted in tallest plant height and the highest values of plant height (cm), chlorophyll (SPAD unit), number of branches/plant, and dry matter (gr/plant) at cuts followed by plant population density 30,000 plants/fed, while the fresh and dry yields (leaves, stems and biological) were recorded with 40,000 plants/fed, these results may be due to the optimum plant population of stevia was 40,000 plant under the study conditions that population gave a higher plants/fed which gave the highest yields. The results are in accordance with the findings of **Murayama *et al*. (1980)** reported also that higher plant height when plant density was adopted (60 cm x 20 cm). **Basuki and Sumaryono (1990)** who reported that an optimum plant density resulted in high crop growth and higher leaf to stem ratio due to higher yield. In addition, **Aladakatti *et a*l. (2011)** found that the closer spacing resulted in higher plant height as compared to wider spacing. As a similar result, **Taleie *et al.* (2012)** also stated that maximum plant height was obtained in the higher plant population density.

The fresh biomass yield, fresh leaf yield, and dry leaf yield at each of the three cuttings, as well as the cumulative yield, varied significantly depending on the planting density. Because there were fewer plants per square foot, the wider spacing could not provide as much dry leaf and biomass as the narrower spacing. Reduced spacing may also improve a crop's capacity to compete and shorten the time that weeds may outcompete crops (**Basuki and Sumaryono, 1990**).

Narrow spacing is a competitive advantage that may result in higher crop yields (**Basuki and Sumaryono, 1990; Kumar *et al*., 2012**). increased dry leaf yield was also found by **Murayama *et al.* (1980)** with increased plant density. **Chalapathi *et al*. (1997)** discovered that stevia planted with 40,000 plants/fed generated better fresh leaf yield and dry leaf yield, following the same general pattern as our findings. According to **Aladakatti *et al*. (2011),** planting geometry with narrow spacing produced the highest overall cumulative dry leaf yield. +Additionally, **Kumar *et al*. (2012)** found comparable results. While **Gvasaliya *et al.* (1990)** found that a spacing of 70 cm 25 cm produced the best stevia growth and production. To maximize crop growth and yield, plant spacing may be employed as a management technique.

In our results, data on stevia's growth and yield characteristics over the three cuts also revealed that the yields were higher in all treatments in the third harvest (cut) compared to the first cut. This occurred because of the hot weather (especially in July and August) and prolonged photoperiod that were prevalent during the crop growth period. As evidenced by the striking increase in production during summer cuttings compared to that of winter cuttings, **Allam *et al.* (2001)** stated that temperature, length, and intensity of photoperiod greatly affected stevia biomass yield. **Lester (1999)** also reported that stevia is sensitive to day duration, photoperiod, and temperature. The timing of harvest is directly connected to yield, according to **Ramesh *et al*. (2007).**

Table (6). Total fresh and dry Leaves, stems and biological yields/Season (t/fed) of stevia plants as affected by salicylic acid (SA), salinity remediator (SR=Cetro- Cal), planting density and their interaction in growing season.

Treatments	Total fresh Leaves yield/season (ton/fed)				Total fresh stems yield/season/(ton/fed)				Biological yield (fresh)/season (ton/fed)			
	B) Plant density/fed											
A)Salicylic acid (SA), salinity remediator (SR=Cetro_Cal)	20000	30000	40000	Average	20000	30000	40000	Average	20000	30000	40000	Average
Control= untreated	1.77	2.34	2.52	2.21	2.23	2.85	3.01	2.70	4.00	5.19	5.53	4.91
Salinity remediator (SR)	2.53	3.09	3.37	3.00	3.02	3.63	3.87	3.51	5.55	6.72	7.24	6.50
Salicylic acid (SA)	2.68	3.09	3.69	3.15	3.35	3.69	4.43	3.82	6.03	6.78	8.12	6.98
SR + SA	2.86	3.63	4.09	3.53	3.45	4.22	4.85	4.17	6.31	7.85	8.94	7.70
Average	2.46	3.04	3.42		3.01	3.60	4.04		5.47	6.64	7.46	
LSD at 0.05 for A		0.23				0.22				0.44		
LSD at 0.05 for B		0.20				0.24				0.42		
LSD at 0.05 for A x B		0.40				0.49				0.84		
Treatments	**Total dry leaves yield/season (ton/fed)**				**Total Dry stems yield/season (ton/fed)**				**Biological yield/season (ton/fed)**			
A)Salicylic acid (SA), salinity remediator (SR=Cetro_Cal)	**B) Plant density/fed**											
	20000	**30000**	**40000**	**Average**	**20000**	**30000**	**40000**	**Average**	**20000**	**30000**	**40000**	**Average**
Control= untreated	0.92	0.94	1.04	0.97	0.98	1.00	1.10	1.03	1.90	1.94	2.14	1.99
Salinity remediator (SR)	1.18	1.36	1.44	1.33	1.24	1.43	1.53	1.40	2.42	2.79	2.97	2.73
Salicylic acid (SA)	1.21	1.34	1.51	1.35	1.29	1.43	1.59	1.44	2.50	2.77	3.10	2.79
SR + SA	1.14	1.4	1.64	1.39	1.21	1.46	1.7	1.46	2.35	2.86	3.34	2.85
Average	1.11	1.26	1.41		1.18	1.33	1.48		2.29	2.59	2.89	
LSD at 0.05 for A		0.05				0.03				0.08		
LSD at 0.05 for B		0.03				0.04				0.07		
LSD at 0.05 for A x B		0.07				0.07				0.14		

SR= Citro- Cal, SA= salicylic acid

The results in **Table 7** revealed the effect of SR, SA, plant density and their interaction on leaf protein content (%) of stevia plants.

In concern to the effect of salinity remediator (SR= Cetro- Cal) and salicylic acid (SA) on protein content in leaves (%), results in Table 7, showed that the significant effect of SR and SA applications on protein %, where the highest values of proteins were recorded with using SR, or SA while the control treatments gave the lowest values. These results are harmony with those recorded by **Kandil (2015); Gomaa *et al.* (2021); Talaat *et al.* (2023)** who reported that application of SR or SA increased protein %.

Regarding the effect of plant density on leaf protein content (%), the results in **Table 7** indicated that there was no significant difference among plant density on this trait.

The same Table cleared that there was significant interaction between the two factors, where the highest value of protein in leaves was obtained with application of SR with 40000 plants/fed.

Table (7). Leaf protein content (%) of stevia plants as affected by salicylic acid (SA), salinity remediator (SR=Cetro_Cal), planting density and their interaction in growing season.

A)Salicylic acid (SA), salinity remediator (SR=Cetro_Cal)	**Leaf protein content (%)**			
	B) Plant density/fed			
	20000	**30000**	**40000**	**Average**
Control= untreated	12.10	10.93	12.37	11.80
Salinity remediator (SR=Cetro- Cal)	12.95	12.28	15.11	13.45
Salicylic acid (SA)	12.50	16.78	11.00	13.43
SR + SA	12.15	11.90	13.60	12.55
Average	12.43	12.97	13.02	
LSD at 0.05 for A		0.66		
LSD at 0.05 for B		ns		
LSD at 0.05 for A x B		1.23		

CONCLUSION

According to the above results of the experiment, it is beneficial to maintain maximum plant density (40,000 plants/fed) with foliar applications of salicylic acid (SA) at a rate of 200 mg/l or salinity remediator (SR=Cetro- Cal) at a rate of 4 litter/fed together or each alone to obtain higher leaf yield/fed and protein content in leaf of stevia. Therefore, this study offered fundamental knowledge on certain stevia agronomy that will be helpful for future research.

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الملخص العربي

استجابة الأستيفيا لحامض السالسليك ومعالج الملوحة والكثافة النباتية تحت ظروف الأرض المتأثرة بالملوحة

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الأستيفيا أو ورقة العسل تعتبر أحدُ كنوز العالم الجديد، موطنُه الأصلي أمريكا الجنوبية، وهو نباتٌ عشبيّ مُعمِّر، تحتوي أوراقه على مجموعة من المواد ذاتِ الطَّعْمِ الحلو التي تفوق نسبة الحلاوة في السكر العادي، وتسمى بـ"الاستيفيوزايدز". له قوةُ تَحليةٍ فائقة، تصل لأكثر من 300 ضعف تحلية السكر العادي المستخرج مِن القصب أو البنجر، والمستخدم في الطعام. ولا تحتوي أوراقُه على سُعرات حرارية، وبالتالي تُعتبَر آمنة ومثالية لتحلية الأطعمة المستخدمة من قِبَل مرضى السكري، وفي برامج التغذية والرشاقة.

تُعتبر أمريكا الجنوبية، خاصة دولتيْ الباراجواي والبرازيل الموطن الأصلي للأستيفيا، حيث يوجد هناك ما يزيد عن 200 نوع نباتي من فصيلة ألـ Stevia.، ولكن نوع rebaudiana هو النوع الذى ينفرد بمحتواه السكري المُركّز "الاستفيوسيد و ريبوديوسيد"، وهي عبارة عن مادة جليكوزيدية فائقة الحلاوة، تركيبُها الكيميائيّ يضم مادتيْ الـ"ستيفيول/ جليكوسايد"، ولا تحتوي على أيّ سُعرات حرارية.

كما أن محصول الأستيفيا من المحاصيل الجديدة في مصر ولا يعرف عن زراعتها الكثير كما انها لا تتحمل الملوحة وبالتالي اقيمت تجارب حقلية من بداية شهر سبتمبر 2022 الى اول شهر سبتمبر 2023 بهدف تحديد الكثافة المناسبة (مسافات الزراعة) لزراعة الأستيفيا وتقليل تأثير الملوحة باستخدام بعض المنشطات الحيوية مثل حامض السالسليك ومعالج الملوحة خاصة للتوسع في زراعة الأستيفيا كأحد محاصيل السكر الجديدة لسد العجز في محاصيل السكر (القصب وبنجر السكر).

وتم تنفيذ المعاملات التجريبية في تصميم القطع المنشقة مرة واحدة في 3 مكررات حيث وزعت المعاملات عشوائيا في القطع الرئيسية: (مقارنة (كنترول) – معالج الملوحة مع مياه الري بمعدل 4 لتر للفدان – الرش الورقي بحمض السالسليك بتركيز 200 مليجم/لتر – الري بمعالج الملوحة بمعدل 4 لتر للفدان والرش الورقي بحمض السالسليك بتركيز 200 مليجم/لتر). بينما القطع الشقية وتتضمن ثلاث كثافات نباتية (20000 و 30000 و 40000 نبات/فدان).
حيث كانت مساحة القطعة التجريبية حوالي 10.5 م^2 تحتوي على 6 خطوط بعرض 50 سم وطول 3.5 م . زرعت النباتات بمسافة (40 ، 26.7 ، 40 سم) على الترتيب.

وتم تلخيص أهم النتائج فيما يلي:

- أثرت المعاملة بمعالج الملوحة والرش الورقي لحامض السالسليك تأثير معنوي على صفات النمو والمحصول تحت الدراسة مثل ارتفاع النبات في ثلاثة فترات حصاد (حشات) ومحتوى الأوراق من الكلوروفيل في كل حشه وعدد الافرع/نبات في كل حشه وتراكم المادة الجافة ومحصول الأوراق الطازج والجاف في الموسم للفدان ومحصول السيقان الطازج والجاف للفدان خلال الموسم والمحصول البيولوجي الجاف والطازج حيث وجد أن الإضافة لمعالج الملوحة والرش بحامض السالسيليك معا أعلى قيم كل الصفات المدروسة يلي ذلك الرش بالسالسيليك منفرداً ثم اضافة معالج الملوحة منفردا مقارنة بالكنترول (بدون معامله) التي بدورها اعطت أقل القيم خلال جميع الحشات في موسم الزراعة 2023 .
- كما أثرت الزراعة بكثافات نباتية مختلفة تأثيراً معنوياً على صفات النمو السابق ذكرها حيث ان الكثافة النباتية الأقل وهي 20000 نبات/الفدان حقق اعلى قيم لصفات النمو للنبات خلال جميع الحشات وتحت ظروف التجربة يليه الزراعة بكثافة

30000 نبات/فدان وكان أقلهم الزراعة بالكثافة الأعلى وهي 4000 نبات/فدان. في حين أن 40000 نبات للفدان سجل اعلى قيم للمحصول للفدان خلال الموسم (للأوراق والسيقان والبيولوجي) الجاف والطازج.

- كما أثر التداخل بين عاملي الدراسة على صفات النمو للنبات حيث وجد ان زراعة الأستيفيا بكثافة 20000 نبات/فدان مع اضافة معالج الملوحة مع مياه الري بمعدل 4 لتر/فدان والرش الورقي بحامض السالسليك بمعدل 200 ملجم/لتر حيث هذه التوليفة حققت أعلى قيم لصفات النمو مقارنة بباقي المعاملات يليها الرش بحامض السالسليك مع نفس الكثافة النباتية ولكن تحت نفس معاملات معالج الملوحة وحمض السالسليك مع كثافة نباتية 40000 حقق أعلى قيم للمحصول للفدان من الأوراق والسيقان والبيولوجي سواء الطازج او الجاف تحت ظروف الدراسة.
- كما ان إضافة معالج الملوحة وحامض السالسليك حقق أعلى نسبة بروتين في الأوراق ولا يوجد تأثير معنوي للكثافات النباتية المختلفة على البروتين في حين ان التداخل بين معالج الملوحة او السالسليك مع 40000 نبات/فدان حققا أعلى نسبة بروتين في الأوراق.

التوصية:

من خلال النتائج السابقة وتحت ظروف ابيس – محافظة الإسكندرية – مصر وجد أنه لتحقيق أعلى محصول وجودة لمحصول الأستيفيا المعمر (5 – 7 سنوات) والزراعة في أرض طينية ثقيلة ومتأثرة بالملوحة يتم زراعة نباتات الأستيفيا بكثافة نباتية عالية تصل الى 40000 نبات/فدان مع الرش الورقي بحامض السالسليك بمعدل 200 مليجم / لتر وإضافة معالج ملوحة (سيتروكال) مع مياه الري بمعدل 4 لتر/فدان خلال موسم النمو كلاهما معاً او أحدهما منفرداً لتحقيق أعلى استفادة من الأرض والتوسع في زراعة الأستيفيا كأحد محاصيل السكر الواعدة لتقليل الفجوة الغذائية من محاصيل السكر (بنجر السكر وقصب السكر) كما وجد ان محصول نباتات الأستيفيا يزداد من حشه لأخرى حتى شهر سبتمبر ويمكن استمرار اخذ حشات اخرى حتى أخر شهر نوفمبر ثم يقل النمو والمحصول حيث تدخل في حالة سكون خلال اشهر الشتاء تحت ظروف المنطقة او المناطق المشابهة كما لابد من اجراء تجارب اخرى مستقبلا للوصول لحزمة توصيات كاملة للمعاملات الخاصة بزراعة الأستيفيا وكيفيه استخراج السكر وزيادة جودته.