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INFLUENCE OF SOME AGRICULTURE TREATMENTS ON GROWTH AND PRODUCTIVITY OF STEVIA (*STEVIA REBAUDIANA* BERTONI) UNDER DESERT LANDS CONDITIONS

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ABSTARCT

A field experiment was conducted in the Northwestern desert of Egypt in the Siwa Oasis region during the 2020/2021 and 2021/2022 seasons to elucidate the influence of plant spacing D1 (50×15 cm), D2 (50×30 cm), and D3 (50×54 cm) combined with biofertilization treatments (*Bacillus megatherium* (B), *Azotobacter chroococcum* (A), *Mycorrhiza* sp. (M), and the mixture between them) on growth and productivity of stevia plant as well as soil microbiological properties.

The results verified that, concerning the effect of interaction, the lowest planting density D3 (50x45 cm = 18600 plants/feddan) with a mix of biostrains (Mycorrhiza, Bacillus, and Azotobacter) recorded a significant increase in plant height, herb fresh and dry weights per plant, total chlorophyll, plant NPK content, soil total microbial count, Azotobacter, Bacillus, and Mycorrhiza counts. While the highest fresh and dry yield per feddan were obtained from the interaction treatment between D1 and the mixture of biostrains. Moreover, the highest soil phosphatase and dehydrogenase activity were noticed from the interaction treatment between D3 and mixture of the three strains. In contrast, the interaction treatment between D2 (28000 plants/feddan) and mix biostrains gave the highest nitrogenase activity and the highest value of stevioside content. While the greatest plant spacing (D3) combined with the mix biostrains (A, B, and M) recorded the highest value in rebaudioside content in the stevia plants.

Key Words: Stevia, Plant spacing, Stevioside, Rebaudioside, Azotobacter, Bacillus, Mycorrhiza.

INTRODUCTION

Stevia rebaudiana Bertoni (Stevia) has economic importance because of the natural sweet compounds called sweet glycosides (SG),

especially stevioside and rebaudioside-A, which are found in its leaves and taste sweet but without calories. The main sweet components in stevia dry leaves are stevioside (STV) (4–13%), which is about 200–300 times sweeter than sucrose, and rebaudioside-A (Reb A) (2–6%), with Reb B and F, as well as dulcoside-A, as minor SG **(Crammer and Ikan, 2003; Tavarini and Angelini, 2013)**. So, sweet glycosides can be used as a natural alternative to synthetic sweeteners viz., aspartame, sucralose, saccharine, asulfam-K that are found in the markets to diabetic people and the diet of obese **(Yadav *et al.,* 2011 and Aladakatti *et al.,* 2012)**. Also, Stevia powder has been reported for hypotensive and heart tonic actions **(Ferri *et al.,* 2006).** At the same time, industry needs large amounts of quality biomass generated with the minimal application of chemical fertilizers.

Sweet glycosides content in stevia leaves greatly depends on the agricultural practices of stevia plants such as planting densities and biofertilizers **(Kumar *et al.,* 2012; Kumar *et al.,* 2013 and Benhmimou *et al.,* 2017)**. Planting density is an important factor for higher production and gives equal opportunity to plants for their survival and best use of other inputs **(Badi *et al.,* 2004 and Benhmimou *et al.,* 2017)**. Optimal density and nutrient available to each plant help to utilize resources (sunlight, water and nutrient) optimally resulting in better yields **(Gomes *et al.,* 2018).**

Biofertilizer have the potential to increase the health and productivity of plant life and reduce the need to use synthetic fertilizers. Most biofertilizers consist of microbes that are involved in the decomposition of organic matter and the breakdown of minerals into a soluble form is useful to plants **(Carvajal-Muñoz & Carmona-Garcia, 2012).** Microorganisms play a central role in the natural N, P and K cycles. The use of N_2-fixers, phosphate and potassium solubilizers contribute in enhancing uptake of plant nutrients **(Afifi *et al.,* 2014)**. Beneficial microorganisms are a tool that enhances plant growth and nutrient uptake.

Azotobacter, phosphate solubilization bacteria and arbuscular mycorrhizal fungi (AMF) are the most generally used as a biofertilizers, increased significantly the soil minerals (N, P, K, Zn, Fe, Cu & Mn) and make them available to the plants. The interaction between the main soil components like minerals, organic matter and microorganisms, shows a great impact on the biological processes of the soils (**Baghat *et al.,* 2022 and Huang, 2002)**.

The beneficial effect of symbiotic nitrogen fixer *Azotobacter chroococcum* as free living N_2-fixing is attributed to fix atmospheric nitrogen, synthesis of phytohormones and vitamins, inhibiting plant ethylene synthesis, enhancing stress resistance and improving nutrient uptake **(Massoud *et al.*, 2013).** Also, they increase the root length, root biomass and better developed root system resulted in an increase in plant growth and yield (**Gupta *et al.*, 2002 ; Shivani *et al.*, 2019; Śniegowska *et al.*, 2024**). Meanwhile, nitrogen is the fourth abundant element in most organisms, can account for as much as 4% of plants dry weight. The majority of nitrogen is present as a continent of protein structure, it is also a component of numerous other biological compounds, such as the chlorophyll and nucleic acids **(Shivani *et al.*, 2019).**

Among all plant growth promoters, Bacillus spp. has been reported to have tolerance towards the adverse conditions and therefore, the most potential candidate is used for enhancing the soil fertility and crop health (**Vivas *et al.*, 2003**). Bacillus spp. is also known to enhance of macro- and micronutrients in the soil and their uptake by host plant (**Stefan *et al.*, 2013).**

Arbuscular mycorrhizal fungi (AMF) have the ability to form symbiotic association with plants that benefit both partners through acquisition and absorption of nutrient especially phosphorus from the soil. AM fungi interact with other soil microbes like free nitrogen fixer the biochemical cycling of elements to the host plants **(Barea *et al.*, 2011 and Soliman *et al.*, 2015)**. On root colonization VAM fungi produce two specialized structures called vesicles and arbuscules in the cortex region of the root. Phosphatases play a major role in the mineralization processes of organic phosphorous and transports of phosphorous which are present in the vacuoles of VAM fungi. Phosphatase plays a main role in the mineralization processes of organic phosphorous and transports the phosphorous which are present in the vacuoles of VAM fungi. Phosphorus is considering an essential nutrient required by plants and microorganisms, its major role in the accumulation and release of energy during cellular metabolism (**Benhmimou *et al.*, 2018**).

Although there are several reports on the microbial inoculants influence on the soil fertility and their support to the plant growth, the synergetic influence of VAM fungi, Azotobacter and PSB on the soil phosphatases activity and nutrients status of the rhizosphere of *Stevia rebaudiana* plant are limited (**Ramakrishnaiah & Vijaya, 2013 and Kumar *et al.*, 2014**), hence the present study was undertaken to evaluate

the soil phosphatases activity and soil nutrients status under the influence of different combinations of bioinoculants.

Therefore, the main goal of the current study was to evaluate the impact of N_2-fixing Azotobacter *chroococcum*, *Bacillus circulanus* as potassium solubilizers, and *Arbuscular mycorrhizal* fungi (AMF) as phosphate solubilizer, individually or in a mixture, on the growth and yield of Stevia under three different plant densities under Siwa Oasis conditions.

MATERIALS AND METHODS

The experiment was implemented from April, 3rd to September, 23rd during the two successive seasons of 2020 and 2021 in a semiarid region in a private farm at Khamisa Village (29° 13' 54.0" N and 25° 23' 38.9" E), Siwa Oasis, Egypt.

The soil of the experimental site was collected from 0 to 30 cm depth before commencement of the experiment and analyzed in at the laboratories of Desert Research Center (DRC). The soil and irrigation water analyses of the experimental farm are presented in Tables (1 and 2) according to **Chapman & Pratt, (1971) ; Page, (1982) and Klute, (1986).** Irrigation was applied through a drip irrigation system with a dripper discharge of 4 L h^{-1}, twice a week. Organic manure at 25 m^3/feddan was added before planting in each season throughout soil preparation. The organic manure analysis is given in Table (3). Also, the meteorological data of two seasons, 2020 and 2021 are shown in table (4).

Table 1. Physical and chemical analysis of the experimental soil site

Depth (cm)		Sand (%)		Silt (%)		Clay (%)		Soil texture	
0-30		93.91		4.32		1.77		Sand	
pH*	E.C. (dS/m)	O.M (%)	Soluble anions (meq/l)				Soluble cations (meq/l)		
			CO_3^{--}	HCO_3^-	Cl^-	SO_4^{--}	Ca^{++}	Mg^{++}	Na^+	K^+
7.6	4.12	0.55	-	3.61	30.42	6.29	8.40	8.50	23.2	0.22

*soil : water suspension (1:2.5)

Table 2. The chemical analysis of irrigation water

pH	E.C. (dS/m)	Soluble anions (meq/l)				Soluble cations (meq/l)			
		CO_3^{--}	HCO_3^-	Cl^-	SO_4^{--}	Ca^{++}	Mg^{++}	Na^+	K^+
7.6	0.59	----	2.09	2.89	0.56	1.59	1.32	2.02	0.61

Table 3. Chemical analysis of the used compost manure.

pH	E.C. (dS/m)	O.M. (%)	C/N ratio	N (%)	P (%)	K (%)	Fe (%)	Mn (mg/kg)	Zn (mg/kg)	Cu (mg/kg)
8.6	4.59	22.6	11.93	1.13	0.25	2.09	3.45	602.8	85.56	42.80

Table 4. Meteorological data at Siwa Oasis during the two seasons.

Parameter / Month	Temp. max. (°C)	Temp.min. (°C)	Rel. hum. (%)	Wind speed (m/s)
Season 2020				
April	26.8	13.5	35.9	4.0
May	32.8	18.3	27.5	3.9
June	35.1	21.4	29.3	3.9
July	35.8	22.3	36.0	3.5
August	37.0	23.2	37.5	3.3
September	35.3	22.0	42.9	3.4
Season 2021				
April	28.8	14.1	29.0	4.1
May	36.2	20.5	23.5	3.8
June	35.3	21.8	30.6	3.7
July	37.2	23.6	33.7	3.7
August	37.8	23.8	35.4	3.7
September	33.8	21.2	43	3.7

Seedlings of stevia were obtained from the Agriculture Research Center, Giza, Egypt. 45 day old stevia seedlings were transplanted in an experimental field on the 3rd and 1st of April for the two seasons, respectively, to study the influence of planting densities and biofertilization treatments on growth, yield components, some chemical constituents of stevia plants, and soil microbiological properties. The experiment consisted of fifteen treatments, which combined between three planting densities of D1 (50cm x 15cm), D2 (50cm x 30 cm), and D3 (50 cm x 45 cm) with a plant population of 56000, 28000, and 18600 plants feddan^{-1}, respectively. Fife bio-fertilization treatments [without strains (control), *Bacillus megaterium* (B), *Azotobacter chroococcum* (A), *Mycorrhiza* sp. (M) and mixture between them (A+B+M)]. The experiment was laid out in a split plot design with three replicates. The main plots involved planting densities, while the sub-plots included biofertilization treatments. The biostrains were used as biofertilizers in the form of single and mixed inoculation at a rate of ~108 CFU/mL.. Biofertilization strains were added twice at the root zone; the first one

was carried out after 15 days from the transplanting date. Meanwhile, the second addition was conducted at a one-month interval after the first one and was carried out again after 15 days from the first cut date. At the same time, all other recommended agricultural practices for growing stevia plants were conducted.

Two cuts were taken per season on July 10th and September 22nd. Harvest was carried out by cutting herb at 10 cm above the soil surface.

The following data were detected for each cut:-

1. Growth parameters:

1.1. Plant height (cm),
1.2. Herb fresh weight / plant(g),
1.3. Herb fresh weight / feddan (Kg),
1.4. Herb dry weight / plant(g),
1.5. Herb dry weight / feddan(Kg)

2. Chemical components:

2.1. **Estimation of total chlorophyll (SPAD)**. Using a Minolta chlorophyll meter (model SPAD 502), the total chlorophyll in plant leaves was measured in SPAD units. Chlorophyll measurements were made using the recently fully expanded leaf and 10 readings were averaged per experimental unit in accordance with **Markwell *et al*. (1995).**

2.2. **Estimation of Stevioside (ppm) and Ruiboside (ppm).** Extraction and identification of stevioside and Ruiboside by HPLC model (Thermo Ultimate 3000). Stevioside samples were extracted according to the method outlined by **Supriyadi *et al.* (2016).** Samples of *Stevia rebaudiana* were powdered and filtered with 20 mesh filter. The powder was later measured to approximately 10 g and put into 250 ml beaker glass. It was then reconstituted with 100 ml of methanol, heated in hot plate50±2 C^{o}, stirred with magnetic stirrers for 15 min, and filtered with filter papers. Much amount of 100 ml of methanol was added to the deposition. Similar processes were repeated 5 times until 500.0 ml of filtrate was extracted and finally ready to analyze by HPLC.

Stevioside and Ruiboside of each sample were identified by comparing their relative retention times with those of the standards mixture chromatogram. The concentration of an individual compound was calculated on the basis of peak area measurements, then converted to µg stevioside g^{-1} dry weight. All chemicals and solvents used were HPLC spectral grade.

2.3. **Determination of NPK in plants.**

The samples of dry herb were wet-digested with H_2SO_4-H_2O_2 digest **(Lowther, 1980)** for measuring total nitrogen, phosphorus, and potassium.

2.3.1. Nitrogen was determined by modified micro-Kjeldahl method.

2.3.2. Phosphorus was determined by using vanado molybdate phosphoric method.

2.3.3. Potassium was measured by Flam-photometer according to **Page *et al.*, (1982).**

3. Soil microbiological properties:

3.1. Determination of total microbial counts in the rhizosphere, the soil samples were collected at harvest stages according to **Bunt and Rovira, (1955).**

3.2. Azotobacter numbers was determined by MPN technique on modified Ashby's medium and calculated using Cochran's tables (**Abd-el-Malek and Ishac, 1968**).

3.3. Bacillus in each treatment was analyzed for their ability to colonize the plant rhizosphere by plate count on Pikovskaya's (PVK) agar medium **(Amri *et al.*, 2023).**

3.4. Arbuscular Mycorrhizal (AMF) in soil was isolated by wet-sieving and decantation method described by **Gerdeman and Nicolson, (1963)**. The plant root colonization percentage and number of spores per gram was carried out according to **Phillips and Hayman, (1970)** staining method.

3.5. Estimate of the VAM infection percentage by using intersect method **(Giovannetti and Mosse, 1980)** using the follow equation:
Root colonization % = No. of positive intersect points / total number of observed intersect points

4. Soil enzymes activity:

4.1. Nitrogenase activity was determined according to **Haahtela, (1985).**

4.2. Phosphatase activity; disodium phenylphosphate served as enzyme substrate **(Öhlinger, 1996).**

4.3. Soil dehydrogenase activity was determined by the reduction of 2,3,5-triphenyltetrazolium chloride (TTC) to 2, 3, 5-triphenylformazan (TPF) **(Serra-Wittling *et al.*, 1995).**

Data obtained in both seasons of study were subjected to analysis of variance as a factorial experiment in split plot design by using XLSTAT software version 2019 (**Addinsoft, 2019**). L.S.D. method was

used to differentiate between means according to **Snedecor and Cochran, (1969)**.

RESULTS AND DISCUSSION

Data presented in Tables (5, 6, and 7) revealed that, the effect of planting spaces, bio-fertilization and their interaction on growth and yield characters of stevia plants. Regarding to the effect of planting density, in the two cuts of both seasons, increasing the spaces between plants within rows from 15 to 45 cm significantly increased plant height, fresh and dry weights of herb per plant but decreased fresh and dry weights of herb per feddan. These results are in harmony with those found by **Kumar *et al.*, (2014) ; Benhmimou *et al.*, (2017) ; Gomes *et al.*, (2018**) on stevia plants who reported that closer plant spacing resulted in higher herb yield per unit area while wider spacing gave higher herb yield per plant which might be attributed to the opportunity of wider-spaced plants to increase the synthesis of metabolites, resulting in growth of more stems and roots.

Table 5. Effect of plant spacing, bio-fertilization and their interaction treatments on plant height of Stevia plants in two seasons.

Treatment	D1	D2	D3	Mean	D1	D2	D3	Means
	1st Cut				2nd Cut			
	Plant height (cm)							
	First season							
Control	25.00 l	28.00 k	30.00 j	27.66 E	29.00 k	34.33 j	36.00 i	33.11 E
Azotobacter (A)	34.33 fg	35.00 ef	35.00 ef	34.77 C	39.00 fg	39.33 fg	40.00 ef	39.44 C
Bacillus (B)	32.00 i	33.00 h	33.66 gh	32.88 D	36.66 hi	37.33 hi	38.00 gh	37.33 D
Mycorrhiza (M)	35.66 de	36.33 d	37.33 c	36.44 B	41.00 de	41.66 cd	43.00 c	41.89 B
A+B+M	38.00 bc	38.66 b	41.66 a	39.44 A	45.00 b	48.66 a	50.00 a	47.89 A
Mean (D)	33.00 C	34.20 B	35.53 A		38.13 C	40.26 B	41.40 A	
	Second season							
Control	30.00 j	31.83 i	32.33 i	31.39 E	33.66 j	37.00 i	39.00 h	36.55 E
Azotobacter (A)	36.00 f	37.00 e	38.00 d	37.00 C	43.66 e	45.00 d	45.00 d	44.55 C
Bacillus (B)	33.67 h	35.00 g	35.33 g	34.67 D	39.66 gh	40.33 g	41.66 f	40.55 D
Mycorrhiza (M)	38.00 d	38.33 d	39.00 c	38.44 B	46.00 cd	46.00 cd	47.00 bc	46.33 B
A+B+M	39.67 b	40.00 b	42.67 a	40.78 A	47.00 bc	47.66 ab	48.33 a	47.66 A
Means (D)	35.47 C	36.43 B	37.47 A		42.00 C	43.20 B	44.20 A	

D= plant spacing, D1= (50*15cm), D2= (50*30cm), D3= (50*45cm),
Means followed by the same letter are not significantly different at $p \leq 0.05$

Table 6. Effect of plant spacing, bio-fertilization and their interaction treatments on herb fresh weight of Stevia plants in two seasons.

Treatment	D1	D2	D3	Means(F)	D1	D2	D3	Means(F)
	Herb fresh weight (g/plant)							
	1st Cut				2nd Cut			
	First season							
Control	24.87 n	36.34 m	44.34 l	35.18 E	60.58 l	67.12 k	73.32 jk	67.01 E
Azotobacter (A)	59.66 h	60.60 h	62.94 g	61.06 C	97.73 gh	99.61 g	103.37 fg	100.24 C
Bacillus (B)	51.37 k	54.64 j	56.73 i	54.25 D	77.42 j	85.14 i	92.56 h	85.04 D
Mycorrhiza (M)	67.29 f	71.53 e	75.62 d	71.48 B	106.94 ef	110.72 e	120.19 d	112.62 B
A+B+M	77.48 c	82.55 b	86.88 a	82.30 A	127.26 c	138.60 b	159.39 a	141.75 A
Means (D)	56.13 C	61.13 B	65.30 A		93.99 C	100.24B	109.77 A	
	Second season							
Control	44.04 m	66.89 l	74.77 k	61.90 E	92.92 m	112.59 l	116.25 kl	107.25 E
Azotobacter (A)	81.08 hi	81.97gh	83.28 g	82.11 C	129.22 h	134.06 g	142.17 f	135.15 C
Bacillus (B)	75.84 k	77.50j	79.82 i	77.72 D	120.03 jk	124.05 ij	125.93 hi	123.34 D
Mycorrhiza (M)	85.95 f	88.58e	92.54 d	89.02 B	149.13 e	151.38 e	174.13 d	158.20 B
A+B+M	97.86 c	106.20 b	118.32 a	107.46 A	192.31 c	200.05 b	221.24 a	204.53 A
Means (D)	76.95 C	84.23 B	89.75 A		136.72 C	144.43 B	155.94 A	
	Herb fresh weight (kg /fed.)							
	First season							
Control	1392.7 j	1017.5 l	824.7 m	1078.3 E	3392.7 f	1879.3 l	1363.8 n	2211.9 E
Azotobacter (A)	3341.0 c	1696.9 g	1170.7 k	2069.5 C	5472.9 c	2789.2 i	1922.7 l	3394.9 C
Bacillus (B)	2876.7 d	1530.0 i	1055.3 l	1820.7 D	4335.5 d	2384.0 j	1721.7 m	2813.7 D
Mycorrhiza (M)	3768.2 b	2003.0 f	1406.7 j	2392.6 B	5988.8 b	3100.0 g	2235.5 k	3774.8 B
A+B+M	4338.9 a	2311.5 e	1616.0 h	2755.5 A	7126.4 a	3880.7 e	2964.7 h	4657.3 A
Means (D)	3143.5 A	1711.8 B	1214.7 C		5263.3 A	2806.6 B	2041.7 C	
	Second season							
Control	2466.2 f	1873.0 i	1390.7 l	1910.0 E	5204.0 f	3153.0 l	2162.0 o	3506.3 E
Azotobacter (A)	4540.3 c	2295.2 g	1549.1 k	2794.8 C	7236.0 c	3754.0 i	2644.0 m	4544.7 C
Bacillus (B)	4247.0 d	2170.1 h	1484.7 k	2633.9 D	6722.0 d	3473.0 j	2342.0 n	4179.0 D
Mycorrhiza (M)	4813.2 b	2480.3 f	1721.3 j	3004.9 B	8351.0 b	4239.0 g	3239.0 k	5276.3 B
A+B+M	5480.2 a	2973.6 e	2200.8 h	3551.5 A	10769.0 a	5601.0 e	4115.0 h	6828.3 A
Means (D)	4309.4 A	2358.4 B	1669.3 C		7656.4 A	4043.9 B	2900.4 C	

D= plant spacing, D1= (50*15cm), D2= (50*30cm), D3= (50*45cm),
Means followed by the same letter are not significantly different at $p \leq 0.05$

Table 7. Effect of plant spacing, bio-fertilization and their interaction treatments on herb dry weight of Stevia plants in two seasons.

Treatment	D1	D2	D3	Means	D1	D2	D3	Means (F)
	Herb dry weight (g/plant)							
	1st Cut				2nd Cut			
	First season							
Control	8.14 n	9.75 m	10.37 l	9.42 E	13.49 l	14.60 kl	15.60 jk	14.56 E
Azotobacter (A)	13.39 h	13.76 g	14.06 fg	13.74 C	17.83 gh	18.50 fg	19.22 f	18.51 C
Bacillus (B)	10.88 k	11.92 j	12.96 i	11.92 D	16.53 ij	17.08 hi	17.56 ghi	17.06 D
Mycorrhiza (M)	14.35 f	14.85 e	15.32 d	14.84 B	20.44 e	21.57 d	22.43 cd	21.48 B
A+B+M	15.85 c	16.32 b	18.42 a	16.86 A	23.12 c	24.57 b	27.69 a	25.13 A
Means (D)	12.52 C	13.32 B	14.22 A		18.28 C	19.26 B	20.50 A	
	Second season							
Control	12.19 l	14.31 k	14.73 jk	13.74 E	19.73 k	20.36 k	22.99 j	21.03 E
Azotobacter (A)	16.35 h	16.86 h	17.47 g	16.89 C	25.74 gh	26.05 gh	26.84 g	26.21 C
Bacillus (B)	18.08 ij	15.35 i	15.58 i	16.34 D	24.54 i	25.10 hi	25.34 hi	24.99 D
Mycorrhiza (M)	18.13 f	18.72 e	20.35 d	19.07 B	29.74 f	31.17 e	32.62 d	31.18 B
A+B+M	21.05 c	21.79 b	22.44 a	21.76 A	35.86 c	38.79 b	41.43 a	38.69 A
Means (D)	17.16 C	17.41 B	18.11 A		27.12 C	28.29 B	29.84 A	
	Herb dry weight (kg /fed.)							
	First season							
Control	455.83 e	273.10 k	192.90 n	307.28 E	755.4 e	408.8 j	290.2 m	484.80 E
Azotobacter (A)	750.20 c	385.47 g	261.60 l	465.76 C	998.3 c	518.1 h	357.4 j	624.61 C
Bacillus (B)	609.67 d	33.97 i	241.07 m	294.90 D	925.5 d	478.3 i	326.6 l	576.81 D
Mycorrhiza (M)	803.77 b	415.93 f	284.87 j	501.52 B	1144.8 b	604.1 g	417.3 j	722.07 B
A+B+M	887.77 a	457.07 e	342.60 h	562.48 A	1294.5 a	688.0 f	515.1 h	832.53 A
Means (D)	701.45 A	313.11 B	264.61 C		1023.7 A	539.5 B	381.3 C	
	Second season							
Control	682.5 e	400.6 jk	273.9 m	452.3 E	1104.9 e	570.2 h	427.7 j	700.9 E
Azotobacter (A)	915.6 c	472.2 h	325.0 l	570.9 C	1441.3 c	729.5 g	499.2 i	890.0 C
Bacillus (B)	844.7 d	429.9 i	289.7 m	521.4 D	1374.4 d	702.9 g	471.4 ij	849.6 D
Mycorrhiza (M)	1015.5 b	524.2 g	378.6 k	639.4 B	1665.6 b	872.8 f	606.8 h	1048.4 B
A+B+M	1179.0 a	610.1 f	417.3 ij	735.5 A	2008.2 a	1086.1 e	770.5 g	1288.3 A
Means (D)	927.4 A	487.4 B	336.9 C		1518.9 A	792.3 B	555.1 C	

D= plant spacing, D1= (50*15cm), D2= (50*30cm), D3= (50*45cm),
Means followed by the same letter are not significantly different at $p \leq 0.05$

As for the effect of biofertilization on growth and yield parameters, in both cuts of both seasons, inoculated plants by all of strains individually or mixture significantly highly significant uninoculated ones in plant height, fresh and dry weights of herb per plant as well as per feddan. The highest values of plant height, herb fresh and herb dry weights were obtained from treatment that (A+B+M), which recorded significant increase compared to other treatments. The increase in herb yield with biofertilization was in agreement with the results reported by **Patil, (2010) ; Vafadar *et al.*, (2014) ; El-Sirafy *et al.*, (2015) and Youssef *et al.*, (2021)** on stevia plant. Regarding the effect of interaction between planting density and biofertilization, in both cuts of both seasons, the lowest planting density D3 (50x45 cm = 18600 plants/feddan) combined with triple strains recorded a significant increase in plant height as well as herb fresh and dry weights per plant. In contrast, the highest planting density D1 (50x15 cm = 56000 plants/feddan) in combination with (A+B+M) demonstrated a significant increase in herb fresh and dry weights per feddan as compared to other interaction treatments. The increment in growth and yield characters by applying the biofertilizer may be due to the effect of different microbial strains of the biofertilizer, such as nitrogen-fixing bacteria (*Azotobacter chroococcum),* which led to nitrogen fixation and the synthesis of vitamins, amino acids, auxins, and gibberellins, which stimulated the plant's growth. Also, phosphate solubilizing bacteria (*Bacillus megaterium*), which is effective on releasing P from inorganic and organic pools of total soil P through solubilizing and mineralization as well as the production of growth-promoting substances. Additionally, AM fungi cause various effects on plants where it is beneficial for the ability to scavenge the available P through their hyphae that have large surface areas on which the extraradical hyphae act as a bridge between the soil and plant roots **(Bianciotto & Bonfante, 2002; Liu *et al.*, 2000).** These results indicate the importance of using biofertilizers as a promising alternative to mineral fertilizers and also consider them an effective tool for desert development and sustainable agriculture.

2. chemical component

2. 1. Total chlorophyll content (SPAD)

With regard to plant spacing factor data in table (8) showed that, D3(18600 plants/ feddan) was the superior treatment on total chlorophyll compared to another two-planting density. In contrast, the lowest result

in this regard achieved from D1 (50cm × 15 cm) compared to other plant spacing.

Table 8. Effect of plant spacing, bio-fertilization and their interaction treatments on total chlorophyll of Stevia plants in two seasons.

Treatment	D1	D2	D3	Means(F)	D1	D2	D3	Means(F)
	Total chlorophyll (SPAD)							
	1st Cut				2nd Cut			
	First season							
Control	33.23 m	36.56 l	37.10 k	35.63 E	37.87 n	38.90 m	39.60 l	38.79 E
Azotobacter (A)	39.33 h	40.03 g	41.23 f	40.20 C	41.17 i	41.67 h	42.17 g	41.67 C
Bacillus (B)	38.20 j	38.76 i	39.10 hi	38.69 D	40.03 k	40.37 k	40.77 j	40.39 D
Mycorrhiza (M)	41.63 f	42.10 e	42.97 d	42.33 B	42.67 f	43.43 e	44.43 d	43.51 B
A+B+M	43.50 c	44.70 b	46.10 a	44.77 A	44.90 c	46.37 b	47.93 a	46.40 A
Means (D)	39.18 C	40.43 B	41.36 A		41.33 C	42.15 B	42.98 A	
	Second season							
Control	35.70 m	38.23 l	41.63 k	38.52 E	41.53 m	43.30 l	44.00 k	42.94 E
Azotobacter (A)	45.30 h	47.07 g	47.67 f	46.68 C	45.73 hi	46.20 h	46.87 g	46.27 C
Bacillus (B)	42.63 j	42.90 j	43.97 i	43.17 D	44.50 jk	45.10 ij	45.30 i	44.97 D
Mycorrhiza (M)	48.37 e	48.97 d	49.60 c	48.98 B	48.23 f	49.00 e	49.93 d	49.05 B
A+B+M	50.00 c	51.83 b	53.07 a	51.63 A	50.63 c	51.57 b	52.27 a	51.49 A
Means (D)	44.40 C	45.80 B	47.19 A		46.13 C	47.03 B	47.67 A	

D= plant spacing, D1= (50*15cm), D2= (50*30cm), D3= (50*45cm),
Means followed by the same letter are not significantly different at $p \leq 0.05$

Concerning microbial soil additives, total chlorophyll is significantly affected by all microbial soil additives. The best results in this regard were achieved with the addition of the mixture between all strains (A+B+M) treatment, followed by Mycorrhiza (M), Azotobacter (A), and then Bacillus (B) treatment. While the control (without strains) treatment recorded the lowest value in this respect compared with other treatments in both cuts of the two seasons. Similar findings were reported by **Vafadar *et al.*, (2014)** on stevia plants.

Relatively to interaction between different factors under study, data indicated that the interaction treatment between D3 and (A+B+M) was the best treatment and gave a significant increase in total chlorophyll in comparison with other interaction ones. Meanwhile, the highest density (D1) combined with the control treatment recorded the worst result in this respect.

2.2. Stevioside content:

The obtained data in Table 9 illustrated that D2 (50×30 cm) treatment was the superior treatment on stevioside content, followed by D1 (50×15 cm) and then D3 (50×45 cm) treatment, which recorded 46.86, 40.17, and 37.69 ppm, respectively. The minimum value of stevioside content was detected by the lowest planting density. These results are in harmony with

those found by **Aladakatti *et al.*, (2012)**; **Benhmimou *et al.*, (2017) and Gomes *et al.*, (2018)** on stevia plants.

With regard to the second factor, data demonstrated that all biofertilization treatments affected stevioside content in stevia plants compared to the control treatment. The addition of mix (A+B+M) was the best treatment in this regard compared to the other four treatments. These results are in agreement with those found by **Portugal *et al.*, (2006) ; Bashan and De Bashan, (2010)** ; **Vafadara *et al.*, (2014) and Aguirre-Medina *et al.*, (2018)** on stevia plants.

As for the interaction effect between plant density and biofertilization treatments, data declared that the plant density D2 (28000 plants/feddan) treated by (A+B+M) achieved the highest value of stevioside content in stevia plants, followed by treatment of D3 combined with the triple strains. However, the treatment of (D3 + control) recorded the lowest value in this respect.

2.3. Rebaudioside content:

The data presented in Table 9 revealed that the highest spacing between plants (D3) was the best treatment on Rebaudioside content of stevia plants, followed by (D2), which recorded 22.62 and 18.65 ppm, respectively. On the other hand, the highest plant density, D1 (50×15 cm) treatment, recorded the lowest value in Rebaudioside content in stevia plants. Regarding the soil microbial addition data showed that the mixture of the three strains (A+B+M) treatment gave the maximum value of Rebaudioside content in stevia plants, which was 32 ppm compared to other biofertilization treatments under this study.

According to the interactions between plant spacing and biofertilization treatments, data declared that the lowest planting space (D1) combined with biofertilization treatment (A+B+M) recorded the highest value in Rebaudioside content of stevia plants, followed by the interaction treatment of D3 and the triple strains. However, the treatment of (D1 + control) recorded the lowest value in this regard.

Table 9. Effect of plant spacing, bio-fertilization and their interaction treatments on total stevioside and rebaudioside of stevia plants during the two seasons (2020 and 2021)

Treatments	D1	D2	D3	Mean	D1	D2	D3	Mean
	Stevioside (ppm)				Rebaudioside (ppm)			
	First season							
Control	14.14 ^{j}	18.47 ^{i}	12.46 ^{k}	15.03 ^{e}	10.02 ^{m}	11.79 ^{jk}	12.54 ^{i}	11.45 ^{e}
Azotobacter (A)	37.34 ^{f}	38.73 ^{ef}	29.66 ^{h}	35.24 ^{c}	11.54 ^{kl}	17.53 ^{f}	26.55 ^{d}	18.54 ^{c}
Bacillus (B)	37.31 ^{f}	29.86 ^{h}	32.74 ^{g}	33.30 ^{d}	11.03 ^{l}	14.32 ^{h}	15.46 ^{g}	13.60 ^{d}
Mycorrhiza (M)	40.32 ^{e}	49.82 ^{d}	40.14 ^{e}	43.43 ^{b}	12.44 ^{ij}	19.30 ^{e}	26.62 ^{d}	19.46 ^{b}
A+B+M	71.74 ^{c}	97.40 ^{a}	73.45 ^{b}	80.87 ^{a}	33.90 ^{a}	30.16 ^{c}	31.95 ^{b}	32.00 ^{a}
Mean	40.17 ^{b}	46.86 ^{a}	37.69 ^{c}		15.79 ^{c}	18.62 ^{b}	22.62 ^{a}	

D= plant spacing, D1= (50*15cm), D2= (50*30cm), D3= (50*45cm)

Means followed by the same letter are not significantly different at $p \leq 0.05$

2.4. Plant nutrient percentage:

Results in Table (10) showed that total nitrogen concentration (total-N) was increased significantly under D3 planting space (1.56 and 1.63%). While the lower concentration was noticed in D1 planting space (1.31 and 1.34%) in two seasons, respectively. The inoculated plants with mixed (A+B+M) under the three planting spaces have significantly recorded the highest total-N followed by Azotobacter and Mycorrhiza treatments when compared with uninoculated ones. At the same time, no significant differences were noticed between Bacillus and control treatments. **(Chen *et al.*, 2018)** stated that the contribution of AM fungi to total-N varies widely in diverse symbiotic systems, but AM fungi can transfer substantial amounts of nitrogen to their hosts. **(Masood *et al.*, 2020)** found that the inoculation of Bacillus improves the growth of tomato under the condition of additional fertilizer N supply due to an increase in N uptake by roots from Bacillus-assisted fixed N in soil.

On the other hand, total-P increased significantly in treatments D2 and D3 when compared to D1 treatment. Total-P concentrations were 0.19 and 0.19; 0.25 and 0.27% for D2 and D3 in two seasons, respectively which increases by 11.8, 11.8; 19.0 and 28.5% when compared to D1 treatment. Also, total-P increased significantly with the inoculation of Mycorrhiza, Bacillus alone or in combination. Where total-P were 0.25 and 0.35; 0.24 and 0.32; and 0.20 and 0.25% for Mix, Mycorrhiza and Bacillus in two seasons, respectively. No significant difference was noticed between Azotobacter and control treatments.

In addition, the D2 and D3 had significantly higher total-K concentration than the D1 treatment with no significant difference between D2 and D3 treatments. The inoculation with Mycorrhiza, Bacillus, Azotobacter individually or mix increased significantly total-K concentration compared to control treatment. Generally, the inoculation of bio-fertilizers Mycorrhiza, Bacillus, Azotobacter and mix significantly improved the NPK percentagein stevia plants compared to the control treatment. The lowest N, P, and K percentages were detected in plants grown in uninoculated treatment. **Govindarajulu *et al.*, (2005)** found that Mycorrhizal fungi are able to deliver enough N for optimal plant growth and development and that inorganic nitrogen taken up by the fungi can be incorporated into amino acids that are further transferred to the plant. Earlier results of studies indicate that Mycorrhizal fungi increase the root surface area that results increasing plant nutrient uptake (**Zhang *et al.*, 2016**). **Vafadar *et al.*, (2014)** studied the effect of inoculation with Bacillus, Pseudomonas, and Azotobacter and Mycorrhizal fungus on stevia. They found that significant increase in NPK uptake by plants. Also, previous studies showing that Mycorrhizal fungi are able to directly assimilate and provide N and K to the host plants **(Toussaint *et al.*, 2004 ; Mortimer *et al.*, 2009 ; Hodge & Storer, 2015)**.

Table 10. Effect of plant spacing, bio-fertilization and their interaction treatments on total-NPK percentage of Stevia plants during the two seasons (2020 and 2021)

Treatment	D1	D2	D3	Mean	D1	D2	D3	Mean
	First season				Second season			
Total-N (%)								
Control	0.79 ^{k}	0.80 ^{k}	0.84 ^{k}	0.81 ^{d}	0.79 ^{k}	0.83 ^{jk}	0.87 ^{j}	0.83 ^{d}
Azotobacter (A)	1.73 ^{e}	1.91 ^{d}	2.01 ^{c}	1.88 ^{b}	1.75 ^{e}	1.98 ^{d}	2.13 ^{c}	1.95 ^{b}
Bacillus (B)	0.85 ^{j}	0.88 ^{j}	0.96 ^{i}	0.90 ^{d}	0.88 ^{i}	0.91 ^{i}	0.98 ^{h}	0.92 ^{d}
Mycorrhiza (M)	1.23 ^{h}	1.45 ^{g}	1.61 ^{f}	1.43 ^{c}	1.29 ^{g}	1.47 ^{f}	1.72 ^{e}	1.49 ^{c}
A+B+M	1.94 ^{d}	2.25 ^{b}	2.39 ^{a}	2.19 ^{a}	1.99 ^{d}	2.28 ^{b}	2.47 ^{a}	2.25 ^{a}
Mean	1.31 ^{c}	1.46 ^{b}	1.56 ^{a}		1.34 ^{c}	1.49 ^{b}	1.63 ^{a}	
Total-P (%)								
Control	0.09 ^{k}	0.10 ^{j}	0.10 ^{j}	0.10 ^{e}	0.10 ^{m}	0.13 ^{l}	0.14 ^{k}	0.12 e
Azotobacter (A)	0.11 ^{i}	0.12 ^{h}	0.14 ^{g}	0.12 ^{d}	0.12 ^{j}	0.13 ^{i}	0.15 ^{g}	0.14 d
Bacillus (B)	0.19 ^{g}	0.20 ^{f}	0.21 ^{e}	0.20 ^{c}	0.20 ^{h}	0.25 ^{g}	0.30 ^{e}	0.25 c
Mycorrhiza (M)	0.21 ^{e}	0.25 ^{c}	0.25 ^{c}	0.24 ^{b}	0.26 ^{f}	0.33 ^{d}	0.36 ^{c}	0.32 b
A+B+M	0.23 ^{d}	0.26 ^{b}	0.27 ^{a}	0.25 ^{a}	0.29 ^{e}	0.39 ^{a}	0.38 ^{b}	0.35 a
Mean	0.17 ^{c}	0.19 ^{a}	0.19 ^{a}		0.21 ^{c}	0.25 ^{a}	0.27 ^{a}	
Total-K (%)								
Control	0.79 ^{k}	0.89 ^{j}	0.82 ^{k}	0.83 ^{e}	0.83 ^{j}	0.95 ^{i}	0.98 ^{i}	0.92 ^{e}
Azotobacter (A)	0.92 ^{j}	1.18 ^{g}	1.22 ^{g}	1.11 ^{d}	0.98 ^{i}	1.26 ^{g}	1.34 ^{f}	1.19 ^{d}
Bacillus (B)	0.97 ^{i}	1.37 ^{e}	1.28 ^{f}	1.21 ^{c}	1.03 ^{h}	1.49 ^{d}	1.44 ^{e}	1.32 ^{c}
Mycorrhiza (M)	1.03 ^{h}	1.45 ^{d}	1.47 ^{d}	1.32 ^{b}	1.07 ^{h}	1.52 ^{d}	1.60 ^{c}	1.40 ^{b}
A+B+M	1.67 ^{c}	1.74 ^{b}	1.79 ^{a}	1.73 ^{a}	1.71 ^{b}	1.92 ^{a}	1.96 ^{a}	1.86 ^{a}
Mean	1.08 ^{b}	1.33 ^{a}	1.32 ^{a}		1.12 ^{c}	1.43 ^{b}	1.46 ^{a}	

D= plant spacing, D1= (50*15cm), D2= (50*30cm), D3= (50*45cm)
Means followed by the same letter are not significantly different at $p \leq 0.05$

3. Soil microbiological properties:

3.1. Total microbial counts:

Rhizosphere are considered the preferred place for microbial community. The fertility and amount of nutrients in the soil were the key factors influencing the microbial communities. As shown in Table (11), the total microbial count increased significantly under D2 planting space followed by D3 and lowest count was noticed under D1 treatment (105.80, 99.63 and 96.40; 121.40, 119.36 and 107.80 x10^{6} cfu /g dry soil) in two seasons, respectively. On the other hand, the inoculation with mix of (A+B+M) gave the highest significant values of total microbial count followed by Mycorrhiza, Bacillus and Azotobacter when compared with control treatment. Total counts were 136.67, 126.33, 95.02 and 82.67; 156.00, 140.00, 115.60 and 99.67 x10^{6} cfu /g dry soil in the two seasons, respectively. These findings are well matched with these obtained by **Kumar *et al.*, (2018)** who studies confirmed the synergistic response between Mycorrhiza, Bacillus, and Azotobacter for different crop species and under field conditions.

3.2. Azotobacter counts:

Data in Table (11) showed that there are high variations of Azotobacter counts between all treatments in Stevia rhizosphere soil in both the two growing seasons. According to planting distance treatments, the highest Azotobacter

counts are recorded with D3 (60.66 and 73.85×10-4 cfu / g dry soil) in two seasons, followed by D2 and D1 (58.40 and 66.20×10-4 cfu / g dry soil) and (51.66 and 57.80×10-4 cfu / g dry soil) in two seasons, respectively. Data of biofertilizer treatments declared that, the highest Azotobacter counts are recorded with mixed biofertilizer treatment (93.00 and 101.67×10-4 cfu / g dry soil) followed by Azotobacter treatment (67.76 and 73.41×10-4 cfu / g dry soil). These data agreed with those found by **Aye, (2011)** who reported that the best results of Azotobacter count produced with the mixture of biofertilizers.

3.3. Bacillus counts:

Data in Table (11) illustrated that the high significantly Bacillus count was noticed under D2 planting space followed by D3 and lowest count was noticed under D1 treatment (35.86, 34.85 and 31.00 $x10^2$ cfu /g dry soil) for first season, respectively. While, the high significantly Bacillus count was noticed under D3 followed by D2 and D1 treatments (44.12, 42.00 and 35.20 $x10^2$ cfu /g dry soil) for second season, respectively. On the other side, the inoculation with mix (A+B+M) gave the highest significant values of bacillus count followed by Bacillus, Mycorrhiza, then Azotobacter when compared with control treatment. Total counts of bacillus were 56.00, 43.33, 31.33 and 26.58; 63.67, 48.00, 38.67 and 43.54 cfu /g dry soil in two seasons, respectively. These findings are well agree with these obtained by **Kumar *et al.*, (2018).**

3.4. Number of spores:

The number of spores per gram soil were 11.89 and 12.46; and 12.04 and 12.30 in the two seasons for D3 and D2 with no significant differences between them. While, significant difference was found between D2 and D1 which has number of spores of 10.94 and 11.34 in two seasons, respectively. Among all inoculation treatments, mixed inoculation of plants had the highest positive effect on spores number. There is significant difference between the mixed treatments (A+B+M), Mycorrhiza and Bacillus, while, no significant difference between Bacillus and Azotobacter treatments. The highest spore number was found in the treatment (A+B+M) followed by mycorrhiza, and Bacillus treatments in two seasons with spores number of 20.83 and 21.33; 17.23 and 17.73; and 8.88 and 8.26% in two seasons, respectively. At the same time, the lowest spores number was found in the control treatment with spores number of 4.27 and 5.20 in two seasons, respectively. These results agreed with those obtained by **Bahadori *et al.*, (2013)** who found that mixed inoculation have a positive effect on increasing root colonization and numbers of VAM spores. Also, **Garbaye (1994)** reported that bacteria produce phytohormones and cohabit in the rhizosphere with VAM fungi and these might stimulate the plant- fungus interaction.

3.5. Mycorrhizal Infection:

Data of mycorrhizal infection (MI) in Table (11) showed that there are significant differences between planting distance treatments and the highest mycorrhizal infection (MI) found in the D3 followed by D2 and D1 treatments in both seasons, with MI percentage of 30.82 and 31.51%; 30.10 and 30.76%; and 28.31 and 28.94% for two seasons, respectively. The results also showed

that, the biofertilizer treatments are significantly differences between the mixed treatments (A+B+M), Mycorrhiza and Bacillus, while there was no significant difference between Bacillus and Azotobacter treatments. The highest MI found in the treatment (A+B+M) followed by mycorrhiza Bacillus treatments in two seasons with MI of 48.68 and 49.57%, 43.93 and 45.27%; and 22.47 and 21.76% in two seasons, respectively. At the same time, the lowest MI was found in the control treatment with MI of 11.77 and 13.17% in two seasons, respectively.

Table 11. Effect of plant spacing, bio-fertilization and their interaction treatments on soil microbiological properties of Stevia plants during the two seasons (2020 and 2021)

Treatment	D1	D2	D3	Mean	D1	D2	D3	Mean
	First season				Second season			
Total microbial count (CFUx10^6 g^{-1} dry soil)								
Control	60.00 ^{i}	64.00 ^{h}	63.10 ^{hi}	62.37 ^{e}	68.00 ^{h}	69.00 ^{h}	72.00 ^{h}	69.67 ^{e}
Azotobacter (A)	81.00 ^{g}	84.00 ^{g}	83.00 ^{g}	82.67 ^{d}	92.00 ^{g}	105.00 ^{ef}	102.00 ^{f}	99.67 ^{d}
Bacillus (B)	96.00 ^{e}	97.00 ^{e}	92.07 ^{f}	95.02 ^{c}	109.00 ^{e}	120.00 ^{cd}	117.81 ^{d}	115.60 ^{c}
Mycorrhiza (M)	115.00 ^{d}	135.00 ^{b}	129.00 ^{c}	126.33 ^{b}	124.00 ^{c}	150.00 ^{b}	146.00 ^{b}	140.00 ^{b}
A+B+M	130.00 ^{c}	149.00 ^{a}	131.00 ^{c}	136.67 ^{a}	146.00 ^{b}	163.00 ^{a}	159.00 ^{a}	156.00 ^{a}
Mean	96.40 ^{c}	105.80 ^{a}	99.63 ^{b}		107.80 ^{c}	121.40 ^{a}	119.36 ^{b}	
Azotobacter (CFUx10^4 g^{-1} dry soil)								
Control	27.00 ^{k}	31.00 ^{j}	30.00 ^{j}	29.33 ^{e}	32.00 ^{k}	39.00 ^{j}	42.00 ^{i}	37.67 ^{e}
Azotobacter (A)	64.00 ^{d}	69.00 ^{c}	70.29 ^{c}	67.76 ^{b}	70.00 ^{e}	74.00 ^{d}	76.23 ^{d}	73.41 ^{b}
Bacillus (B)	36.30 ^{i}	42.00 ^{h}	46.00 ^{g}	41.43 ^{d}	41.00 ^{ij}	51.00 ^{h}	60.00 ^{f}	50.67 ^{d}
Mycorrhiza (M)	46.00 ^{g}	54.00 ^{f}	59.00 ^{e}	53.00 ^{c}	57.00 ^{g}	60.00 ^{f}	82.00 ^{c}	66.33 ^{c}
A+B+M	85.00 ^{b}	96.00 ^{a}	98.00 ^{a}	93.00 ^{a}	89.00 ^{b}	107.00 ^{a}	109.00 ^{a}	101.67 ^{a}
Mean	51.66 ^{c}	58.40 ^{b}	60.66 ^{a}		57.80 ^{c}	66.20 ^{b}	73.85 ^{a}	
Bacillus (CFUx10^2 g^{-1} dry soil)								
Control	12.00 ^{i}	12.30 ^{i}	12.50 ^{i}	12.27 ^{e}	15.00 ^{k}	18.00 ^{j}	19.00 ^{j}	17.33 ^{e}
Azotobacter (A)	24.00 ^{h}	29.00 ^{f}	26.73 ^{g}	26.58 ^{d}	29.00 ^{i}	37.00 ^{h}	37.62 ^{h}	34.54 ^{d}
Bacillus (B)	39.00 ^{d}	46.00 ^{c}	45.00 ^{c}	43.33 ^{b}	43.00 ^{fg}	49.00 ^{e}	52.00 ^{d}	48.00 ^{b}
Mycorrhiza (M)	27.00 ^{g}	34.00 ^{e}	33.00 ^{e}	31.33 ^{c}	30.00 ^{i}	42.00 ^{g}	44.00 ^{f}	38.67 ^{c}
A+B+M	53.00 ^{b}	58.00 ^{a}	57.00 ^{a}	56.00 ^{a}	59.00 ^{c}	64.00 ^{b}	68.00 ^{a}	63.67 ^{a}
Mean	31.00 ^{c}	35.86 ^{a}	34.85 ^{b}		35.20 ^{c}	42.00 ^{b}	44.12 ^{a}	
No. of Spores g^{-1} dry soil								
Control	3.80 ^{i}	4.00 ^{i}	5.00 ^{hi}	4.27 ^{d}	4.20 ^{j}	5.30 ^{ij}	6.10 ^{hij}	5.20 ^{d}
Azotobacter (A)	6.70 ^{ghi}	6.80 ^{gh}	7.20 ^{g}	6.90 ^{c}	7.20 ^{ghi}	7.70 ^{gh}	8.00 ^{g}	7.63 ^{c}
Bacillus (B)	8.10 ^{g}	9.70 ^{f}	8.83 ^{f}	8.88 ^{c}	8.00 ^{g}	8.30 ^{f}	8.50 ^{f}	8.26 ^{c}
Mycorrhiza (M)	17.00 ^{e}	17.40 ^{d}	17.30 ^{d}	17.23 ^{b}	18.00 ^{e}	17.40 ^{d}	17.80 ^{d}	17.73 ^{b}
A+B+M	19.10 ^{c}	22.30 ^{a}	21.10 ^{b}	20.83 ^{a}	19.30 ^{c}	22.80 ^{a}	21.90 ^{b}	21.33 ^{a}
Mean	10.94 ^{b}	12.04 ^{a}	11.89 ^{a}		11.34 ^{b}	12.30 ^{a}	12.46 ^{a}	
Mycorrhizal Infection (%)								
Control	11.30 ^{i}	12.00 ^{i}	12.00 ^{i}	11.77 ^{d}	12.50 ^{i}	13.10 ^{hi}	13.90 ^{h}	13.17 ^{d}
Azotobacter (A)	20.00 ^{h}	22.80 ^{f}	22.77 ^{f}	21.86 ^{c}	20.80 ^{g}	23.00 ^{f}	22.97 ^{f}	22.26 ^{c}
Bacillus (B)	21.20 ^{g}	22.70 ^{f}	23.51 ^{f}	22.47 ^{c}	21.00 ^{g}	21.80 ^{fg}	22.47 ^{f}	21.76 ^{c}
Mycorrhiza (M)	41.80 ^{e}	44.00 ^{d}	46.00 ^{c}	43.93 ^{b}	42.30 ^{e}	46.40 ^{d}	47.10 ^{cd}	45.27 ^{b}
A+B+M	47.24 ^{b}	49.00 ^{a}	49.80 ^{a}	48.68 ^{a}	48.10 ^{c}	49.50 ^{b}	51.10 ^{a}	49.57 ^{a}
Mean	28.31 ^{c}	30.10 ^{b}	30.82 ^{a}		28.94 ^{c}	30.76 ^{b}	31.51 ^{a}	

D= plant spacing, D1= (50*15cm), D2= (50*30cm), D3= (50*45cm), CFU – colony forming units,

Means followed by the same letter are not significantly different at $p \leq 0.05$

4. **Soil enzymes activity**:

4.1. **Nitrogenase enzyme**:

Data in Table (12) illustrated that, nitrogenase enzyme is regarded as an indication of the ability free living Azotobacter to fix atmospheric nitrogen and transfer it to nitrate. The Nitrogenase enzyme activity increased significantly in D2 planting space treatment. With D2, the activity reached its optimum values were recorded 105.80 and 109.88 µmole C_2H_4 g^{-1}soil h^{-1} in two seasons, respectively. The enzyme activity decreased under D3 and D1 treatments, their values 99.73 and 102.89; 97.20 and 100.62 µmole C_2H_4 g^{-1}soil h^{-1} in two seasons, respectively. Biofertilizers treatments increased significantly the Nitrogenase activity where the mix (A+B+M) treatment records the highly activity followed by Azotobacter, Mycorrhiza and Bacillus when compared with control treatment. The activities were 136.67, 126.33, 95.02 and 82.83; 140.80, 129.33, 99.05 and 85.10 µmole C_2H_4 g^{-1}soil h^{-1} in two seasons, respectively. Since some PGPRs are thought to be able to convert nitrogen into ammonia in a free state due to the nitrogenase enzyme complex, ammonia concentration has a significant effect on nitrogen expression in the majority of diazotophs **(Afifi *et al.*, 2014).** Nitrogen fixing Azotobacter could fix atmospheric nitrogen by nitrogenase enzyme, that is considered a sign of microbial activity. This process of converting the nitrogen to ammonia and then to nitrate is the only form readily available to uptake by plants (**Baldani & Baldani, 2005).**

4.2. Phosphatase and dehydrogenase enzymes:

The data presented in the same Table (12) show that, the activity of phosphatase and dehydrogenase enzymes in soil were highly significant under D3 planting space which records 0.44 and 0.47 mg PNP g^{-1} soil for phosphatase and 35.36 and 36.46 TPF µg g^{-1} d^{-1} for dehydrogenase, in two seasons, respectively. While D2 and D1 followed the D3 in phosphatase and dehydrogenase enzymes activity. On the other side, biological fertilizers increase the activity of phosphatase and dehydrogenase enzymes. The highly activity of phosphatase was noticed in mix (A+B+M) treatment followed by mycorrhiza, bacillus and azotobacter when compared with control. The activity of phosphatase enzyme was 0.58, 0.51, 0.49 and 0.34; 0.61, 0.55, 0.52 and 0.36 mg PNP g^{-1} soil for two seasons, respectively. While, the activity of dehydrogenase enzyme was 54.65, 48.52, 29.28 and 22.73; 56.77, 50.34, 29.97 and 23.12 TPF µg g^{-1} d^{-1} for two seasons, respectively. These results were agreed with results obtained by **Shaimaa & Massoud,**

(2017). The increase of phosphatase enzyme was due to the strong symbiotic relationship between mycorrhiza, Azotobacter and bacillus which could solubilize complex inorganic phosphorus forms through producing some organic acids and release phosphatase enzyme that help in solubilizing low sources of phosphorus **(Aloni *et al.,* 2006).** Simultaneously, dehydrogenase is an oxidoreductase, which only present in viable cells and is improve of soil health and is a valid indicator of changes in total microbial count in soil management **(Roldán *et al.,* 2004).** The increase of activity of this enzyme with low amount of mineral nitrogen and phosphorus was due to the increase of viable microbial population in the rhizosphere of some plants and the effect of microbial strains **(Afifi *et al.,* 2014).**

Table 12. Effect of plant spacing, bio-fertilization and their interaction treatments on soil enzymes activity of Stevia plants during the two seasons (2020 and 2021)

Treatment	D1	D2	D3	Mean	D1	D2	D3	Mean
	First season				Second season			
Nitrogenase (µmole C_2H_4 g^{-1} Soil h^{-1})								
Control	64.00 ^{h}	64.00 ^{h}	63.10 ^{h}	63.70 ^{e}	69.40 ^{j}	70.10 ^{j}	64.60 ^{k}	68.03 ^{e}
Azotobacter (A)	115.00 ^{d}	135.00 ^{b}	129.00 ^{c}	126.33 ^{b}	116.00 ^{d}	139.00 ^{b}	133.00 ^{c}	129.33 ^{b}
Bacillus (B)	81.00 ^{g}	84.00 ^{g}	83.50 ^{g}	82.83 ^{d}	82.60 ^{i}	85.90 ^{hi}	86.80 ^{h}	85.10 ^{d}
Mycorrhiza (M)	96.00 ^{e}	97.00 ^{e}	92.07 ^{f}	95.02 ^{c}	99.10 ^{f}	104.00 ^{e}	94.05 ^{g}	99.05 ^{c}
A+B+M	130.00 ^{c}	149.00 ^{a}	131.00 ^{c}	136.67 ^{a}	136.00 ^{bc}	150.40 ^{a}	136.00 ^{bc}	140.80 ^{a}
Mean	97.20 ^{c}	105.80 ^{a}	99.73 ^{b}		100.62 ^{c}	109.88 ^{a}	102.89 ^{b}	
Phosphatase (mg PNP g^{-1} soil)								
Control	0.19 ^{ij}	0.18 ^{j}	0.20 ^{i}	0.19 ^{e}	0.20 ^{k}	0.19 ^{k}	0.24 ^{j}	0.21 ^{e}
Azotobacter (A)	0.32 ^{h}	0.33 ^{h}	0.37 ^{g}	0.34 ^{d}	0.33 ^{i}	0.37 ^{h}	0.39 ^{g}	0.36 ^{d}
Bacillus (B)	0.47 ^{f}	0.49 ^{ef}	0.52 ^{d}	0.49 ^{c}	0.50 ^{f}	0.53 ^{e}	0.52 ^{ef}	0.52 ^{c}
Mycorrhiza (M)	0.49 ^{e}	0.50 ^{e}	0.54 ^{c}	0.51 ^{b}	0.52 ^{e}	0.56 ^{d}	0.57 ^{cd}	0.55 ^{b}
A+B+M	0.56 ^{b}	0.58 ^{a}	0.59 ^{a}	0.58 ^{a}	0.58 ^{c}	0.63 ^{a}	0.61 ^{b}	0.61 ^{a}
Mean	0.41 ^{c}	0.42 ^{b}	0.44 ^{a}		0.43 ^{c}	0.46 ^{b}	0.47 ^{a}	
Dehydrogenase (TPF µg g^{-1} d^{-1})								
Control	5.93 ^{j}	6.28 ^{j}	6.28 ^{j}	6.16 ^{e}	5.97 ^{j}	7.51 ^{i}	7.41 ^{i}	6.96 ^{e}
Azotobacter (A)	17.36 ^{i}	18.92 ^{h}	31.90 ^{f}	22.73 ^{d}	17.52 ^{h}	19.84 ^{g}	32.00 ^{e}	23.12 ^{d}
Bacillus (B)	24.21 ^{g}	31.90 ^{f}	31.73 ^{f}	29.28 ^{c}	24.29 ^{f}	32.80 ^{e}	32.82 ^{e}	29.97 ^{c}
Mycorrhiza (M)	46.57 ^{e}	49.30 ^{d}	49.70 ^{d}	48.52 ^{b}	47.61 ^{d}	52.50 ^{b}	50.90 ^{c}	50.34 ^{b}
A+B+M	51.29 ^{c}	55.48 ^{b}	57.19 ^{a}	54.65 ^{a}	53.00 ^{b}	58.15 ^{a}	59.15 ^{a}	56.77 ^{a}
Mean	29.07 ^{c}	32.38 ^{b}	35.36 ^{a}		29.68 ^{c}	34.16 ^{b}	36.46 ^{a}	

D= plant spacing, D1= (50*15cm), D2= (50*30cm), D3= (50*45cm)

Means followed by the same letter are not significantly different at $p \leq 0.05$

CONCLUSION

Planting space and biofertilizers as a better supplement are able to improve growth, nutrient status, microbial count, enzymes activity and stevioside and Rebaudioside content of stevia plants. Regarding the

effect of interaction, the lowest planting density D3 (50x45cm=18600 plants/feddan) with mix biofertilizers (mycorrhiza, bacillus and azotobacter) recorded significantly highest plant height, fresh and dry weights of herb per plant. The interaction treatment between D3 and mix biofertilizers gave a significant increase in total chlorophyll, plant NPK content, total microbial count, azotobacter, bacillus and mycorrhiza count. Also, the highest phosphatase and dehydrogenase activity were notices under D3 and mix biofertilizers treatments. In contrast, the D2 (28000 plants/feddan) and mix biofertilizers gave the highest nitrogenase activity. Treatment D2 with mix biofertilizers achieved the highest value of stevioside content followed by treatment of D2 combined with Mycorrhiza. While, the lowest plant spacing (D3) combined with mix biofertilization recorded the highest value in Rebaudioside content followed by D3 with Mycorrhiza.

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تأثير بعض المعاملات الزراعية على نمو وانتاجية نبات الإستيفيا تحت ظروف الأراضى الصحراوية

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أجريت تجربة حقلية في صحراء شمال غرب مصر بمنطقة واحة سيوة خلال موسمي 2021/2020 و2022/2021 لتوضيح تأثير مسافات الزراعة (50 × 15 سم) D1 و (50 × 30 سم) D2 و (50 × 45 سم) D3 مع معاملات التسميد الحيوي (*Azotobacter chroococcum* (A) ، و *Bacillus megatherium* (B) ، و *Mycorrhiza* sp. (M) والخليط بينهم) على نمو وإنتاجية نباتات الاستيفيا وكذلك الخصائص الميكروبيولوجية للتربة. وقد أثبتت النتائج فيما يتعلق بتأثير التفاعل أن أقل كثافة زراعة D3 (50 × 45سم = 18600 نبات/فدان) مع خليط من السلالات الحيوية (*Azotobacter* ، *Bacillus* ، *Mycorrhiza*) سجلت زيادة معنوية في إرتفاع النبات، و وزن العشب الطازج والجاف للنبات، والكلوروفيل الكلي، ومحتوى النبات من العناصر الغذائية الأساسية (NPK)، وعدد الميكروبات الكلية في التربة، وعدد الآزوتوباكتر، والباسيلس، والميكروهيزا. في حين تم الحصول على أعلى محصول طازج وجاف للفدان من معاملة التفاعل بين D1 وخليط السلالات الحيوية. علاوة على ذلك لوحظ أعلى نشاط للفوسفاتيز والديهيدروجينيز في التربة من معاملة التفاعل بين D3 والأسمدة الحيوية المختلطة. وعلى النقيض من ذلك، أعطت معاملة D2 (28000 نبات/فدان) والأسمدة الحيوية المختلطة أعلى نشاط للنيتروجينيز وأعلى قيمة لمحتوى ستيفيوسيد. بينما سجلت أكبر مسافة بين النباتات (D3) الممزوجة مع السلالات الحيوية المختلطة (A و B وM) أعلى قيمة في محتوى الريبوديوسيد في نباتات الاستيفيا.