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The Effect Of Planting Date, Adding Of Mycorrhiza, Bio-Stimulators, And Interactions Among Them In The Accumulation Of Some Active Compounds In The Different Parts Of *Stevia rebaudiana* Bertoni. Plant Cultivated In Iraq.

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ABSTRACT

The experiment was conducted in one of the plastic house in the research station B of Department of Horticulture and Gardening Landscape, College of Agricultural Engineering Sciences, University of Baghdad for the spring season 2017. The experiment was conducted according to the Randomized Complete Block Design (RCBD) in the order of Split-Split Plot Design, with three replicates. The experiment included the planting date factor in the main plots, with two planting dates (15 and 30 March). In the sub-plots, the inoculation treatment with Mycorrhiza fungal (adding the Mycorrhiza fungal to the root system or without adding it). In the sub-sub-plots included five treatments of bio-stimulators are: The control treatment, The addition treatment of chemical fertilizer (NPK) to the ground, the foliar spraying treatment on total vegetative with Chitosan at concentration of (2 $ml.L^{-1}$), the foliar spraying treatment on total vegetative with bread yeast extract (4 $g.L^{-1}$) and the foliar spraying treatment on total vegetative with seaweed extract at concentration of (1 $ml.L^{-1}$). The averages for all study indicators were compared by a least significant difference (LSD) at the 5% probability level. The results showed that the triple interaction treatment between the studied factors had the greatest effect by giving the highest percentages for each of Stevioside and Rebaudioside A and total Steviol glycosides. The triple interaction treatment among of the first planting date, the addition of the fungal Mycorrhiza vaccine to the root and the spraying of total vegetative with Chitosan (D1M1C2) was excelled by giving it the highest percentage for each of Stevioside compound and Rebaudioside A and total Steviol glycosides in all dried plant parts (leaves, stems, branches and roots) of S.rebaudianaBertoni. plant, which amounted (8.82%, 4.36%, 13.18%, 0.640%, 0.397%, 1.037%, 0.240%, 0.127%, 0.367%), respectively, compared to the control treatment (D2M0C0) which recorded a decrease in the percentage of the same compounds in all dried plant parts (leaves and stems with branches and roots), amounted of (3.77%, 1.23%, 5.01%, 0.160%, 0.110%, 0.270%, 0.030%, 0.010, 0.040%), respectively.

Keyword: Stevia plant , date time , bio fertilizer chitosan, seaweed extract and yeast extract .

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INTRODUCTION

One of the main commercially aims for breeders and producers of medicinal plants is obtaining medicinal plants with a high content of bioactive substances, known as secondary compounds (Zhao et al., 2005). The production of secondary compounds in plants is affected by several factors, including biofertilizers (Vassilev et al., 2015; Akhzari et al., 2016) and bio-stimulators (Cheng et al., 2013; Rafiee et al., 2016), as well as planting date of the plant (Khan et al., 2012). The transfer date of plants to the permanent field leads a significant role in determining the quantitative and qualitative traits for medicinal plants (Khan et al., 2012) because of the environmental conditions prevailing, which positively or negatively reflect on the formation of active compounds (Maheshwar, 2005; Khan et al., 2012). The using of microorganisms in the increase agricultural production and improve its quality is considered one of the modern technologies introduced into the agricultural field on a large scale. Mycorrhiza is one of the most important microorganisms living in the surrounded region with roots, which is known as rhizosphere (Kloepper et al., 1990). The Mycorrhiza fungus is characterized by its symbiotic with the roots of vascular plants (Malusà et al., 2016). The vaccination of the roots of some medicinal plants with Mycorrhiza fungus works to make increase in the growth and content of these plants from secondary compounds because of what its provide of the symbiotic relationship between the medicinal plant and Mycorrhiza fungus from increase in the absorption of nutrient elements as a result of the high capacity of Mycorrhiza to exploit simple organic nitrogen sources, which contributes to the promoting of plant growth as well as what the inoculation with Mycorrhiza provides from water and nutrient elements, as well as improving the structure of the soil (Warburton-Egerton et al., 2008; Heil, 2011; Sadhana , 2014), which is positively reflected in improving the growth and content of medicinal plants from total secondary metabolites (Araim et al., 2009; Sharafzadeh, Zare, 2011; Akhzari et al., 2016). While the action mechanism of Bio-stimulators, which create naturally as amino acids, yeast extract, seaweed extracts, yeast extract, seaweed extracts, chitosan, essential elements, hormones and plant growth regulators, are shown in the light of various mechanisms to working as nitrogen assimilation, the using of Sustainable agriculture in the production of medicinal plants, and the increase in bio-mass production and improve the synthesis of secondary metabolites. This has been demonstrated by numerous experiments and studies in vitro and in-vivo, which have examined the effect of these bio-stimulators on the production of active compounds in medicinal plants (Rafiee et al., 2016). Stevia rebaudianaBertoni is considered one of the important medicinal plants belonging to the Asteraceae family (Mubarak et al., 2008). South America, specifically east of Paraguay and Brazil, is considered the original home of this plant (Ahmed et al., 2011). The medicinal significance of Stevia rebaudianaBertoni plant is due to its highly leaves content of a group of very sweet compounds free from calories, called the steviol glycosides, which are extracted and purified from leaves of this plant. Among the most important compounds that steviol glycosides included , Steviosids compound is a Diterpene glycosides consisting of three molecules: glucose, glycol and steviol. The most important compounds associated with Stevioside include Steviol, Rebaudioside A, Isosteviol and Dihydroisosteviol (Varanuj and Chatachai, 2009). Rebaudioside A is considered the second most important compound of the steviol glycosides group with medical importance in this plant (Farooqi, Sreeramu, 2004 and Ahmed et al., 2011). The medical significance of Stevioside and Rebaudioside A is due to the many pharmaceutical and therapeutic properties they possess (Goyal, 2010, Pemba and Sharangi, 2016), due to the importance of this plant, it is necessary to increase its growth and production by adopting environmentally friendly fertilization systems whose use does not cause damage or side effects, whether on the plant or the environment. Because of the absence of previous studies of the cultivation of Stevia rebaudianaBertoni. plant in Iraq and the importance of medicinal plants and the important role of these factors in growth and production as a Quantitatively and qualitatively, this study aims to demonstrate the effect of planting date, adding of Mycorrhiza, bio-stimulators, and interactions among them in the accumulation of some active medical compounds in the different parts of Stevia rebaudianaBertoni. plant cultivated in Iraq.

MATERIALS AND METHODS

The experiment was conducted in one of the plastic house in the research station B of Department of Horticulture and Gardening Landscape, College of Agricultural Engineering Sciences, University of Baghdad for the spring season 2017 according to the Randomized Complete Block Design (RCBD) in the order of Split-Split Plot Design, with three replicates where resulted from treatments and their replicates 60 experimental units. The soil of the plastic house was prepared from conducting tillage, smoothing, and leveling. The soil was divided into a width of 0.50 m and a height of 0.30 cm and a length of 9 m. A distance of 4 m was left between the raws and 1 m between the experimental units within the single raw. Nine samples were taken from

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different locations of the house soil. The analysis was carried out to determine the physical, chemical and biological traits for the soil of plastic house as shown in Table (1). The seedling was obtained from the plants of tissue culture for Siembra primavera. cultivar. The seedlings were cultivated in seedling trays and then were adapted inside Lath house prepared for this purpose until they became seedlings at the age of 6 weeks. Transfer process for those cultivated seedlings were conducted from inside the plastic trays to the land of the plastic house where the plants were planted with double lines. The distance between one plant and another within the single line was 0.20 m. The drip irrigation system was used for irrigation and the agricultural service operations were conducted in a uniform manner for all treatments. The location was equipped with a device to measure the temperature and humidity inside the protected house and the non-woven polypropylene spun bounded (with thickness 17 GSM and 3% UV) was added with height 2.5 cm from the plant (Santosh et al., 2017) to reduce the temperature and solar radiation As shown in Table (2), the house was equipped with a device to measure soil temperature inside the house.

Table 1: Physical, chemical and biological traits for the soil of plastic house.

Soil texture	Soil separates (%)			pH	EC ($ds.m^{-1}$)	K availability ($mg.kg^{-1}$)	P availability ($mg.kg^{-1}$)	N availability ($mg.kg^{-1}$)	organic matter ($g.kg^{-1}$)	Ca (ml equivalent.L^{-1})
	Clay	Sand	Silt							
SandyLoam	12.4	75.6	12.0	7.31	1.83	75	5.13	33	2.9	9.91
	Mg (ml equivalent.L^{-1})		Na (ml equivalent.L^{-1})	K (ml equivalent.L^{-1})	Cl(ml equivalent.L^{-1})	HCO_3^- (ml equivalent.L^{-1})	CO_3^- (ml equivalent.L^{-1})	SO_3^- (ml equivalent.L^{-1})	$CaCO_3$ (ml equivalent.L^{-1})	CEC ($cmol.kg^{-1}$)
	4.06		3.41	0.75	15.73	1.31	Nil	1.62	182.1	12.41
	Total Fungi CFU ($g.dry\ soil^{-1}$)				Total Bacteria CFU ($g.dry\ soil^{-1}$)		* Types of bacteria found in the soil of the plastic house		Azotobacter sp.	
									Psedomonas sp.	
									Bacilliussp	
	3.1×10^4				6.2×10^5		* Types of fungi found in the soil of the plastic house		Aspergillusflorecens	
									Pencilleum sp.	

Table 2: Maximum and minimum temperatures (C), relative humidity (%) inside and outside the protected house and the average number of hours of solar brightness outside the protected house and the temperature of the soil inside the protected house.

Inside the protected house						** Outside the protected house					
Month	Days	Maximum temperature (C)	Minimum temperature (C)	Relative Humidity (%)	Soil temperature inside the house	Month	Days	Maximum temperature (C)	Minimum temperature (C)	Relative Humidity (%)	Average number of hours of solar brightness
March	2*	24.5	14.2	68.2	25	March	2	22.2	12.8	55.5	**7.2**
	3*	27.5	15.1	62.2	25		3	23.6	12.3	57.4	
April	1*	32.6	16.5	58.9	26	April	1	27.2	13.6	47.2	**7.9**
	2	30.9	18.4	59.4	26		2	31	17.1	47.6	
	3	31.6	19.9	39.9	26		3	34	16.1	28.3	
May	1	33.8	20.9	38.1	26.5	May	1	36.9	20.7	29.9	**10.9**
	2	36.1	23.7	35.4	28		2	39.9	22.5	22.1	

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	3	35.2	26.1	36.7	27.5		3	37.6	20.6	25.4	
June	1	38.1	27.3	35.1	28	June	1	41.8	22.3	22.2	**12.8**
	2	40.1	28.1	33.2	28.5		2	42.7	25.8	22	
	3	40.5	29.6	29.8	28.7		3	43.8	26.6	19.1	

* (1) represents the average of the first ten days of the month.
* (2) represents the average of the second ten days of the month.
* (3) represents the average of ten to eleven days, the third of the month and according to the Gregorian calendar.
** The maximum and minimum temperatures, relative humidity and solar brightness outside the protected house from the Ministry of Transport and Communications / Department of Meteorological in Baghdad for the year 2017 of station 650.

The experiment included three factors. The planting dates factor represents the main plots with two planting dates (15, 30) March, which is symbolized by (D1, D2). The sub-plots include the inoculation treatment with fungal Mycorrhiza by two treatments: The adding of the Mycorrhiza fungal vaccine to the root system or without adding it which is symbolized by (M1, M0). A 15 g.plant^{-1} of Mycorrhiza fungus (Glomusmossea) in pit in contact with the roots of the seedlings with the addition (250 g.plant^{-1}) of sterilized peat moss according to the method mentioned by (Abd El-Fattah et al., 2013). The number of spores for Mycorrhiza vaccine loaded on the soil (51 spore.g^{-1} soil). The sub-sub-plots include the bio-stimulators, with five treatments: the control treatment and The addition treatment of chemical fertilizer (NPK) to the ground where this fertilizer was added as recommended by (ALadakatti et al., 2012) with some modification in the percentage of phosphorus element where the amount of phosphorus was reduced from 150 to 100 kg.ha^{-1} for presence the treatment with the Mycorrhiza fungus, the NPK availability (Joudsmartfert) is given with a fertilizer combination (300: 100: 100 Kg.ha^{-1}). Table (3) shows the components of the fertilizer combination of NPK. Which divided into batches along the plant growth stages. The foliar spraying treatment on total vegetative with Chitosan at concentration of (2 ml.L^{-1}), The first spraying was given after the first week of cultivation of seedlings, and the remaining three sprayings were repeated after every 15 days during the growing season (Saif-Eldeen et al., 2014). Table (4) shows the content of the Chitosan in the plant care fertilizer produced by Al-Khdraa Reef, and the foliar spraying treatment on total vegetative with bread yeast extract at a concentration of (4 g.L^{-1}). The treatment was done by spraying the first spraying after one month of cultivating the seedlings in the protected house. The second spraying was given to the plants after month of the first spraying (Salama et al., 2016). The bread yeast extract was prepared in the fungus laboratory belonging to the Medical and Aromatic Plants Research Unit according to the method described by (Chalutz et al.,1977) by taking weight of 4 g and dissolve it in a liter of distilled water (water temperature 32 °C) with the addition of 1: 1 sugar with the aim of activating the yeast. The conical flask containing a solution of dissolved yeast in water and sugar was placed in vibrator device for a full hour to accelerate the process of flipping the solution and thus activate the yeast and release its internal contents into the solution. Then put the volumetric flask in the incubator at 25 °C for 24 hours, then remove the volumetric flask containing the solution from the incubator. The solution containing the dry bread yeast extract was filtered by a piece of Acrylic cloth. Table (5) shows the ingredients of the dry bread yeast extract and produced by Turkey's Lesaffre Company, according to the analysis of the components of bread yeast by (Al-Dulaimi, 2012) and the spraying treatment of the total vegetable with seaweed extract at a concentration of (1 ml.L^{-1}) where it was added to plants specified to the treatment of seaweed, with two sprayings: The first spraying was one month after cultivating the seedlings in the protected house. The second spraying was given to the plants after one month of the first spraying (Salama et al., 2016). Table (8) shows the components of seaweed extract, the treatments are symbolized by (C0, C1, C2, C3, C4). The results were statistically analyzed by use of the statistical program Gestate and the averages of all traits were compared according testing the least significant difference (LSD) at the 5% probability level (Al-rawi and Khalaf Allah, 1980).

Table 3: Sources of active elements constituents for JoudSmartFert fertilizer

Element	its composition	Element	its composition	Element	its composition
N	Urea	P	P_2O_5	K	K_2O

Table 4: Components of Plant Care Liquid Fertilizer and produced by Al-Khdraa Reef.

Active components	Proportion	Active components	Proportion
Chitosan (multiple amino acids)	25 ($g.L^{-1}$)	Total Nitrogen	50 ($g.L^{-1}$)
Organic matter, vitamins and micro-elements	14 ($g.L^{-1}$)	P_2O_5	40 ($g.L^{-1}$)

Table 5: Ingredients of dry bread yeast (Sacchromycecervisiae) produced by Turkish company Lesaffre (Al-Dulaimi, 2012).

No.	Amino Acids ($mg.g^{-1}$)		2-	K	(%)	0.18
1-	Glycine	0.103	3-	Na		0.12
2-	Alanine	0.132	4-	Mg		0.10
3-	Valine	0.312	5-	Ca		0.04
4-	Leucine	0.067	6-	Mn	($mg.g^{-1}$)	5.69
5-	Isoleucine	0.421	7-	Zn		69.5
6-	Aspartic acid	0.274	8-	Cu		12.78
7-	Glutamic acid	0.367	9-	Fe		30.5
8-	Serine	0.523	No.	Vitamins ($mg.g^{-1}$)		
9-	Threonine	0.206	1-	Vit.B1		0.163
10-	Tyrosine	0.031	2-	Vit.B2		0.054
11-	Phenyl alanine	0.116	3-	Vit.B6		0.019
12-	Proline	0.041	4-	Pantothenic acid		0.058
13-	Arginine	0.073	5-	Biotin		0.091
14-	Lysine	0.089	6-	Niacin		0.112
15-	Cysteine	0.025	7-	Inositol		0.372
16-	Methionine	0.012	No.	Other ingredients (%)		
17-	Histidine	0.078	1-	Total Nitrogen		7.69
18-	Tryptophan	0.020	2-	Carbohydrate		5.47
No.	Mineral Composition		3-	Ash		13.51
1-	P%	0.94	4-	Water		4.7
No.	Amino Acids ($mg.g^{-1}$)		2-	K	(%)	0.18
1-	Glycine	0.103	3-	Na		0.12
2-	Alanine	0.132	4-	Mg		0.10
3-	Valine	0.312	5-	Ca		0.04
4-	Leucine	0.067	6-	Mn	($mg.g^{-1}$)	5.69
5-	Isoleucine	0.421	7-	Zn		69.5
6-	Aspartic acid	0.274	8-	Cu		12.78
7-	Glutamic acid	0.367	9-	Fe		30.5
8-	Serine	0.523	No.	Vitamins ($mg.g^{-1}$)		
9-	Threonine	0.206	1-	Vit.B1		0.163
10-	Tyrosine	0.031	2-	Vit.B2		0.054
11-	Phenyl alanine	0.116	3-	Vit.B6		0.019
12-	Proline	0.041	4-	Pantothenic acid		0.058
13-	Arginine	0.073	5-	Biotin		0.091
14-	Lysine	0.089	6-	Niacin		0.112
15-	Cysteine	0.025	7-	Inositol		0.372
16-	Methionine	0.012	No.	Other ingredients (%)		
17-	Histidine	0.078	1-	Total Nitrogen		7.69
18-	Tryptophan	0.020	2-	Carbohydrate		5.47
No.	Mineral Composition		3-	Ash		13.51
1-	P%	0.94	4-	Water		4.7

Studied traits:

Estimation of medically effective compounds in different parts of S.rebaudianaBertoni. plant:

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Estimation of the leaves content of branches, stems and roots from Stevioside and Rebaudioside A plant:

A concentration of Stevioside and Rebaudioside A was evaluated at the Agricultural Research Center (ARC) belonging to the Ministry of Agriculture and Land Reclamation in the Arab Republic of Egypt using a High Chromotography Performance Liquid HPLC acorrding to (Vaněk et al., 2001), it included the following steps:

Extraction of Stevioside and Rebaudioside A compounds and its separation:

The plant parts, which included leaf and stems with branches and roots, were separated after the plant harvesting process in the evening for the purpose of conducting an analysis to two compounds of Stevioside and Rebaudioside A. When the leaves reached full expansion stage and before the flowering, after the completion of the spraying treatments with bio-stimulators by ten days, dried each of the plant parts, which included: leaves, branches, stems and roots at the room temperature until the stability of weight and then grind the samples using a special industrial mill For this purpose, the samples were kept in sealed paper bags, which were also placed in sealed Polyethylene bags, such as Zipper frezzer bags (Falcon type), and placed in the refrigerator until the time of analysis at 4-5 °C. Sample solution was prepared as shown in chart (1). A standard solution from the Stevioside compound imported from Latoxan company and Rebaudioside A compound imported from Sigma-Aldrich company was used. This process was repeated with samples of both branches, stems, and roots.

The plant sample solution was prepared by taking 2 g of crushed plant specimen for leaves, stems, root and branches for each separately and it was placed in 50 ml of boiled distilled water and left for 30 seconds.

↓

Leave the water extract to cool

↓

The organic layer (methanol) was withdrawn from the extract by the rotary evaporator, the dry residue was collected and 1 mL of methanol was added to dissolve the dry sample

↓

The solution was filtered with filter paper 0.45 µm

↓

The solution was filtered with a filtration filter of 0.45 mµ

↓

The filtrate was then taken into the HPLC.

Chart 1: Steps for preparing the plant sample (leaves, stems, branches, roots).

Device conditions:

The percentage of Stevioside and Rebaudioside A compounds was estimated for Stevia rebaudianBertoni plant in the laboratories of the National Center for Agricultural Research, Food Technology Research Institute, Egypt. The chromatography method was applied to estimate the concentration of Stevioside and Rebaudioside A in leaves, branches and stems, roots. The HPLC device (Agilent Model 1200) is used to determine the retention time and the sample area for both the standard solution and the sample solution. The column (C18 type) was used with dimensions (7µ × 4mm × 250mm). The mobile phase, which started with batch (15 acetonitrile : 75 water) and end with batch (50 water: 50 acetonitrile) in 30 minutes with a running rate of (1.0 $min.ml^{-1}$). The readings were measured along a wavelength of 205 nm and at a temperature of 35 °C. A standard solution of Stevioside and Rebaudioside A was used and then measure the compound in the standard models by comparing the peaks area of the model with the area of the known peaks of the required standard material and repeat the process on all the samples that were diagnosed under the same separation conditions. When comparing the unknown peak with the standard peak, the stevioside and Rebaudioside A. were diagnosed. The concentration of compounds in the model was calculated according to the following Calibration equation:

$$\text{Model concentration in sample } (mg.g^{-1}) = \frac{\text{Standard model concentration} \times \text{Model peak area} \times \text{Number of dilution times}}{\text{standard Area of model peak}}$$

The concentration of each of the active compounds in all the models was then determined and then converted to percentage (Vaněk et al., 2001).

Percentage of Total Steviol glycosides in leaves, stems, branches and roots of plant:

It was calculated by sum a percentage of the Stevioside compound with A Rebaudiosides compound in each of the following parts: Leaves and stems with the branches and roots of plants treatment that were measured and then calculated the average.

RESULTS AND DISCUSSION

Estimate the percentage of Stevioside in dried leaves of plant (%):

Table (7) shows a significant effect of most of the study factors individually in estimating the percentage of the Stevioside compound in the dried leaves of the plant under study.The M1 treatment was excelled by recording it the highest percentage of Stevioside in leaves compared to M0 treatment, which recorded the lowest percentage of the same compound, which amounted of (6.76%, 5.26%), respectively.The C2 treatment has excelled on C0 treatment by giving it the highest percentage of Stevioside in leaves amounted of (6.97%), while C0 treatment showed a decrease in the percentage of Stevioside compound (4.36%).While D1 and D2 did not show significant differences between them as shown in Table (7). The Bi-interaction treatments between planting dates and fungal Mycorrhiza vaccine (D × M), planting dates and bio-stimulators (D × C), and fungal Mycorrhiza vaccine and bio-stimulators (M × C) showed significant differences in percentage of Stevioside in dried leaves of S. rebaudianaBertoni. plant. The bi-interaction treatments (D1M1, D2M1, D1C2, D1C4, M1C2) were excelled recording them the highest percentage of Stevioside compound (6.98%, 6.56%, 7.27%, 7.23%, 8.45%), respectively.while the bi-interaction treatments (D2M0, D2C1, D1C0, D2C0, M0C1, M1C0, M0C0) recorded the lowest percentages of the Stevioside compound: (4.91%, 5.40%, 4.66%, 4.07%, 5.00%, 4.54%, 4.19%), respectively, as shown in Table (7).The interaction treatment D1M1C2 has characterized by recording it the highest percentage of Stevoside (8.82%) compared to the D2M0C0 treatment which recorded the lowest percentage of Stevoside (3.77 %).

Table 7: Effect of planting dates, Mycorrhiza and bio-stimulators in percentage of Stevioside compound in dried leaves of S. rebaudianaBertoni. plant.

Planting dates	Mycorrhiza vaccine	Bio-stimulators					Interaction D x M
		C0	C1	C2	C3	C4	
D1	M0	4.60	5.30	5.72	5.82	6.63	5.61
	M1	4.72	6.57	8.82	6.95	7.83	6.98
D2	M0	3.77	4.69	5.26	5.33	5.50	4.91
	M1	4.36	6.11	8.08	6.72	7.48	6.55
							Effect of planting dates D
Interaction (Planting dates x Bio-stimulators) D x C	D1	4.66	5.94	7.27	6.39	7.23	6.29
	D2	4.07	5.40	6.67	6.03	6.49	5.73
							Effect of fungal Mycorrhiza vaccine M
Interaction (fungal Mycorrhiza vaccine x Bio-stimulators) M x C	M0	4.19	5.00	5.49	5.58	6.07	5.26
	M1	4.54	6.34	8.45	6.84	7.65	6.76
Effect of Bio-stimulators C		4.36	5.67	6.97	6.21	6.86	
L.S.D 0.05							
D×M×C	M×C	D×C	D × M	C	M		D
2.243	1.586	1.586	1.577	1.122	1.115		N.S

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Estimation the percentage of Rebaudioside A in dried leaves of plant (%):

Table (8) shows a significant effect of most of the study factors individually when estimating the percentage of Rebaudioside A in the dried leaves of the plant under study.The M1 treatment was excelled by recording the highest percentage of Rebaudioside A amounted of (3.15%) compared to M0 treatment, which recorded the lowest percentagefor the same compound of (2.25%). Both C2 and C4 treatments recorded the highest percentage of Rebaudioside A which reached of (3.26%, 3.15%), respectively. While both C1 and C0 treatments recorded the lowest percentage for the same compound of (2.41%, 1.73%), respectively. No significant differences were found between treatments D1 and D2 as shown in Table (8).The Bi-interaction treatments between planting dates and fungal Mycorrhiza vaccine (D × M), planting dates and bio-stimulators (D × C), and fungal Mycorrhiza vaccine and bio-stimulators (M × C) showed significant differences in the percentage of Rebaudioside A in dried leaves of S .rebaudianaBertoni. plant.The bi-interaction treatments (D1C1, D1C2, D1C4, D1C3, D2C2, D2C4, M1C2) were excelled by giving it the highest percentage of Rebaudioside A which amounted of (3.41%, 3.42%, 3.26%, 3.09%, 3.09%, 3.03%, 4.16%), respectively.while D2M0, D2C1, D1C0, D2C0 and M0C0 treatments recorded the lowest percentage of Rebaudioside A which amounted (2.10%, 2.02%, 1.96%, 1.50%, 1.52%), respectively, as shown in Table (8).The triple interaction treatment (D × M × C) was significantly differed between study factors in the percentage of Rebaudioside A in the dried leaves of S.rebaudianaBertoni. plant.The triple interaction treatment (D1M1C2) was characterized by recording it the highest percentage of Rebaudioside A (4.36%) compared to the triple interaction treatment (D2M0C0), which recorded the lowest percentage of Rebaudioside A (1.23%), respectively as shown in Table (8).

Table 8: Effect of planting dates, Mycorrhiza and bio-stimulators in the percentage of Rebaudioside A compound in dried leaves of S. rebaudianaBertoni. plant.

Planting dates	Mycorrhiza vaccine	Bio-stimulators					Interaction D x M
		C0	C1	C2	C3	C4	
D1	M0	1.80	2.34	2.49	2.67	2.70	2.40
	M1	2.10	3.26	4.36	3.52	3.82	3.41
D2	M0	1.23	1.93	2.22	2.43	2.67	2.10
	M1	1.78	2.11	3.96	3.19	3.40	2.89
							Effect of planting dates D
Interaction (Planting dates x Bio-stimulators) D x C	D1	1.96	2.80	3.42	3.09	3.26	2.91
	D2	1.50	2.02	3.09	2.81	3.03	2.49
							Effect of fungal Mycorrhiza vaccine M
Interaction (fungal Mycorrhiza vaccine x Bio-stimulators) M x C	M0	1.52	2.13	2.35	2.55	2.69	2.25
	M1	1.94	2.68	4.16	3.35	3.61	3.15
Effect of Bio-stimulators C		1.73	2.41	3.26	2.95	3.15	
L.S.D 0.05							
D×M×C	M×C	D×C	D × M	C	M		D
1.423	1.006	1.006	1.244	0.711	0.880		N.S

Total percentage of Steviol glycosides in dried leaves of plant (%):

Table (9) shows that there is a significant effect of most of the study factors individually in the total percentage of Steviol glycosides in the dried leaves of the S.rebaudianaBertoni. plant.The treatments (M1, C2, C4) recorded the highest percentage of Steviol glycosides in the dried leaves of the plant under study, which amounted of (9.91%, 10.22%, 10.01%), respectively.While M0 and C0 treatments recorded a decrease in the total percentage of Steviol glycosides which reached (7.51%, 6.10%), respectively.No significant differences were found between the first planting date D1 and the second planting date D2 as shown in Table (9). The Bi-interaction treatments between planting dates and fungal Mycorrhiza vaccine (D × M), planting dates and bio-

stimulators (D × C), and fungal Mycorrhiza vaccine and bio-stimulators (M × C) showed significant differences in the total percentage of Steviol glycosides in the dried leaves of S.rebaudianaBertoni. plant.The bi-interaction treatments (D1M1, D1C2, D1C4, D2C2, D2C4, M1C2) were excelled by giving it (10.39%, 10.68%, 10.49%, 9.76%, 9.52%, 12.61%), respectively, while the bi-interaction treatments (D1M0, D2M0, D2C1, D1C0, D2C0, M0C0) gave (8.01%, 7.01%, 7.42%, 6.62%, 5.57%, 5.72%), respectively, as shown in Table (9).The triple interaction treatment (D × M × C) was significantly differed between study factors in the total percentage of Steviol glycosides in the dried leaves of S.rebaudianaBertoni. plant.The triple interaction treatments (D1M1C2, D2M1C2) was characterized by recording it the highest total percentage of Steviol glycosides in the dried leaves of S.rebaudianaBertoni. plant which amounted of (13.18%, 12.04%), respectively. While the triple interaction treatments (D1M1C0, D2M0C1, D1M0C0, D2M1C0, D2M0C0) recorded a significant decrease in the total percentage of Steviol glycosides in the dried leaves of S.rebaudianaBertoni. plant which reached (6.82 6.62%, 6.42% 6.14%, 5.01%), respectively as shown in Table (9).

Table 9: Effect of planting dates, Mycorrhiza and bio-stimulators in the percentage of Steviol glycosides in the dried leaves of S.rebaudianaBertoni. plant

Planting dates	Mycorrhiza vaccine	Bio-stimulators					Interaction D x M
		C0	C1	C2	C3	C4	
D1	M0	6.42	7.64	8.19	8.49	9.33	8.01
	M1	6.82	9.83	13.18	10.46	11.64	10.39
D2	M0	5.01	6.62	7.48	7.77	8.17	7.01
	M1	6.14	8.22	12.04	9.91	10.87	9.44
							Effect of planting dates D
Interaction (Planting dates x Bio-stimulators) D x C	D1	6.62	8.73	10.68	9.48	10.49	9.20
	D2	5.57	7.42	9.76	8.84	9.52	8.22
							Effect of fungal Mycorrhiza vaccine M
Interaction (fungal Mycorrhiza vaccine x Bio-stimulators) M x C	M0	5.72	7.13	7.84	8.13	8.75	7.51
	M1	6.48	9.02	12.61	10.19	11.26	9.91
Effect of Bio-stimulators C		6.10	8.08	10.22	9.16	10.01	
L.S.D 0.05							
D×M×C	M×C	D×C	D × M	C	M	D	
2.953	2.088	2.088	1.730	1.476	1.223	N.S	

Estimate the percentage of Stevioside in dried branches and stems of plant (%):

Table (10) shows a significant effect of most of the study factors individually when estimating the percentage of the Stevioside compound in the dried branches and stems of the plant under study.The M1, C2 and C4 treatments were excelled by recording it the highest the percentage of the Stevioside compound which amounted of (0.352%, 0.436%, 0.345%), respectively. while M0, C1 and C0 treatments showed a significant decrease in percentage of the same compound in dried branches and stems of the plant under study(0.222%,0.212%,0.168% respectively).There were also no significant differences between planting dates (Table 10). The Bi-interaction treatments between planting dates and fungal Mycorrhiza vaccine (D × M), planting dates and bio-stimulators (D × C), and fungal Mycorrhiza vaccine and bio-stimulators (M × C) showed significant differences in percentage of Stevioside in dried branches and stems of S. rebaudianaBertoni. plant.The bi-interaction treatments (D1M1, D1C2, M1C2) were excelled recording them the highest percentage of Stevioside compound (0.364%, 0.445%, 0.622%), respectively. while the bi-interaction treatments (D2M0, D1C1, D2C2, D1C0, D2C0, M0C2 M0C1, M1C0, M0C0) showed a significant decrease in the percentages of the Stevioside compound of (0.210%, 0.213%, 0.207%, 0.170%, 0.165%, 0.230%, 0.180%, 0.172%, 0.163%), respectively, as shown in Table (10). The triple interaction treatment (D × M × C) was significantly differed between study factors in the percentage of Stevoside in dried branches and stems of S. rebaudianaBertoni. plant.The triple interaction treatment (D1M1C2) was characterized by recording it the highest percentage of stevioside which amounted of (0.640%) compared to the D2M0C0 interaction treatment which recorded a significant decrease in the percentage of the same compound which reached of (0.160%) as shown in Table (10).

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Table 10: Effect of planting dates, Mycorrhiza and bio-stimulators in the in percentage of Stevioside in dried branches and stems of S. rebaudianaBertoni. plant.

Planting dates	Mycorrhiza vaccine	Bio-stimulators					Interaction D x M
		C0	C1	C2	C3	C4	
D1	M0	0.167	0.183	0.250	0.273	0.300	0.235
	M1	0.173	0.243	0.640	0.323	0.440	0.364
D2	M0	0.160	0.177	0.210	0.250	0.253	0.210
	M1	0.170	0.237	0.603	0.300	0.383	0.339
							Effect of planting dates D
Interaction (Planting dates x Bio-stimulators) D x C	D1	0.170	0.213	0.445	0.298	0.370	0.299
	D2	0.165	0.207	0.407	0.275	0.318	0.274
							Effect of fungal Mycorrhiza vaccine M
Interaction (fungal Mycorrhiza vaccine x Bio-stimulators) M x C	M0	0.163	0.180	0.230	0.262	0.277	0.222
	M1	0.172	0.240	0.622	0.312	0.412	0.352
Effect of Bio-stimulators C		0.168	0.212	0.436	0.287	0.345	
L.S.D 0.05							
D×M×C	M×C	D×C	D × M	C	M		D
0.2189	0.2096	0.2096	0.1512	0.1504	0.1069		N.S

Estimation the percentage of Rebaudioside A in dried branches and stems of plant (%):

Table (11) shows a significant effect of most of the study factors individually when estimating the percentage of Rebaudioside A in the dried branches and stems of the plant under study. The treatment (M1 and C2) was excelled by recording it the highest percentage of Rebaudioside A amounted of (0.228% and 0.268%) respectively, while the treatments (M0, C1, C0) recorded the lowest percentage for the same compound, which reached (0.147%, 0.149%, 0.115%), respectively, as shown in Table (11).The Bi-interaction treatments between planting dates and fungal Mycorrhiza vaccine (D × M), planting dates and bio-stimulators (D × C), and fungal Mycorrhiza vaccine and bio-stimulators (M × C) were significantly affected in the percentage of Rebaudioside A in dried branches and stems of S .rebaudianaBertoni. plant.The bi-interaction treatments (D1M1, D1C2, M1C2) were excelled by giving it the highest percentage of Rebaudioside A which amounted of (0.246%, 0.275%, 0.389%), respectively. while D2M0, D1C0, D2C0 and M0C0 treatments recorded the lowest percentage of Rebaudioside A which amounted (0.141%, 0.118%, 0.112%, 0.113%), respectively, as shown in Table (11).It was characterized by the treatment of interference triple D1M1C2 recorded the highest percentage of the compound Rebaudioside A, reaching 0.397%, while each of the overlapping triangular transactions recorded D1M0C4 and D2M0C3 and D1M0C4 and D1M1C1 and D1M0C3 and D2M0C3 and D1M0C2 and D2M1C1 and D1M0C1 and D2M0C2 and D2M0C1 and D1M1C0 and D1M0C0 and D2M1C0 and D2M0C0 the lowest percentage of the same compound which amounted of (0.193%, 0.193, 0.113%, 0.120%, 0.113%, 0.120%, 0.117%, 0.113%, 0.113%, 0.113%, 0.153%, 0.153%, 0.153%, 0.153%, 0.153%).

Table 11: Effect of planting dates, Mycorrhiza and bio-stimulators in the percentage of Rebaudioside A in the dried branches and stems of S. rebaudianaBertoni. plant.

Planting dates	Mycorrhiza vaccine	Bio-stimulators					Interaction D x M
		C0	C1	C2	C3	C4	
D1	M0	0.117	0.143	0.153	0.160	0.193	0.153
	M1	0.120	0.177	0.257	0.257	0.280	0.246
D2	M0	0.110	0.123	0.153	0.153	0.187	0.141
	M1	0.113	0.153	0.193	0.193	0.213	0.211
							Effect of planting dates D
Interaction (Planting dates x Bio-stimulators) D x C	D1	0.118	0.160	0.275	0.209	0.237	0.200
	D2	0.112	0.138	0.260	0.173	0.200	0.176
							Effect of fungal Mycorrhiza vaccine M

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Interaction (fungal Mycorrhiza vaccine x Bio-stimulators) M x C	M0	0.113	0.133	0.147	0.157	0.190	0.147
	M1	0.117	0.165	0.389	0.225	0.247	0.228
Effect of Bio-stimulators C		0.115	0.149	0.268	0.191	0.219	
L.S.D 0.05							
D×M×C	M×C	D×C	D × M	C	M		D
0.1961	0.1305	0.1305	0.0878	0.0981	0.0621		N.S

Total percentage of Steviol glycosides in dried branches and stems of plant (%):

Table (12) shows that there is a significant effect of most of the study factors individually in the total percentage of Steviol glycosides in the dried branches and stems of the S.rebaudianaBertoni. plant.The treatments (M1, C2) recorded the highest percentage of Steviol glycosides in the dried branches and stems of the plant which amounted of (0.580%, 0.694%), respectively. While observed a decrease in the total percentage of Steviol glycosides in the branches and stems in both treatments (M0 and C0), which amounted (0.288%, 0.370%), respectively. There were no significant differences between planting dates as shown in Table (12).The Bi-interaction treatments between planting dates and fungal Mycorrhiza vaccine (D × M), planting dates and bio-stimulators (D × C), and fungal Mycorrhiza vaccine and bio-stimulators (M × C) affected significant differences in the total percentage of Steviol glycosides in the dried branches and stems of S.rebaudianaBertoni. plant.The bi-interaction treatments (D1M1,D2C4,D1C4,D2C2,D1C2, D1C3, M1C2) were excelled by giving it the highest total percentage of Steviol glycosides (0.610%, 0.720%, 0.667%,0.607,0.518,0.507,1.011%), respectively, while the bi-interaction treatments (D1M0, D2M0, D2C3, D1C1, D2C1, D1C0, D2C0, M0C2, M0C1, M1C0, M0C0) showed a significant decrease in the total percentage of Steviol glycosides, which amounted of (0.388%, 0.353%, 0.448%, 0.373%, 0.345%, 0.289%, 0.277 %, 0.377%, 0.313%, 0.288%, 0.277%), respectively as shown in Table (12).The triple interaction treatment (D × M × C) was significantly differed between study factors in the total percentage of Steviol glycosides in the dried branches and dried stems of S.rebaudianaBertoni. plant. The triple interaction treatments (D1M1C2, D2M1C2) was characterized by recording it the highest total percentage of Steviol glycosides in the dried branches and stems of S.rebaudianaBertoni. plant which amounted of (1.037%, 0.984%), respectively., compared to the D2M1C4 D1M1C3, D1M0C4, D2M1C3, D2M0C4, D1M1C1, D1M0C2, D2M0C3, D2M1C1, D2M0C2, D1M0C1, D2M0C1, D1M1C0, D1M0C0, D2M1C0, and D2M0C0 treatments which recorded the lowest total of the percentage of Steviol glycosides which amounted (0.420%, 0.433%, 0.440%, 0.493%, 0.493, 0.580, 0.596%, 0.403%, 0.403%, 0.390%, 0.350%, 0.326%, 0.300%, 0.293%, 0.284%, 0.283%0.270%), respectively as shown in Table (12).

Table 12: Effect of planting dates, Mycorrhiza and bio-stimulators in the total percentage of Steviol glycosides in the dried branches and stems of S. rebaudianaBertoni. plant.

Planting dates	Mycorrhiza vaccine	Bio-stimulators					Interaction D x M
		C0	C1	C2	C3	C4	
D1	M0	0.283	0.377	0.403	0.433	0.493	0.388
	M1	0.327	0.420	1.037	0.580	0.720	0.610
D2	M0	0.270	0.307	0.350	0.397	0.440	0.353
	M1	0.283	0.390	0.984	0.493	0.596	0.549
							Effect of planting dates D
Interaction (Planting dates x Bio-stimulators) D x C	D1	0.289	0.373	0.720	0.507	0.607	0.499
	D2	0.277	0.345	0.667	0.448	0.518	0.451
							Effect of fungal Mycorrhiza vaccine M
Interaction (fungal Mycorrhiza vaccine x Bio-stimulators) M x C	M0	0.277	0.312	0.377	0.415	0.467	0.370
	M1	0.288	0.405	1.027	0.537	0.658	0.580
Effect of Bio-stimulators C		0.258	0.359	0.694	0.478	0.563	
L.S.D 0.05							
D×M×C	M×C	D×C	D × M	C	M		D
0.3656	0.2585	0.2585	0.0568	0.1828	0.0402		N.S

Estimate the percentage of Stevioside in dried roots of plant (%):

Table (13) shows a significant effect of most of the study factors individually when estimating the percentage of the Stevioside compound in the dried roots of the plant under study. The M1, C2 treatments were excelled by recording it the highest the percentage of the Stevioside compound which amounted of (0.125%, 0.152%), respectively, compared to M0 and C0 treatments, which recorded the lowest percentage of the same compound, which amounted of (0.069%, 0.042%), respectively. Planting dates showed no significant differences in the same traits as shown in Table (13). The Bi-interaction treatments between planting dates and fungal Mycorrhiza vaccine (D × M), planting dates and bio-stimulators (D × C), and fungal Mycorrhiza vaccine and bio-stimulators (M × C) showed significant differences in percentage of Stevioside in dried roots of S. rebaudianaBertoni. plant. The bi-interaction treatments (D1M1, D2M1, D1C2, M1C2) were excelled by recording them the highest percentage of Stevioside compound (0.130%, 0.120%, 0.159%, 0.232%), respectively. While the bi-interaction treatments (D1M0, D2M0, D1C3, D2C3, D1C1, D2C1, D1C1, D2C1, D1C0, D2C0, M0C0) recorded the lowest percentages of the Stevioside compound of (0.072%, 0.065%, 0.099%, 0.092%, 0.072%, 0.068%, 0.045%, 0.039%, 0.032%), respectively, as shown in Table (13).The triple interaction treatment (D × M × C) was significantly differed between study factors in the percentage of Stevoside in dried roots of S. rebaudianaBertoni. plant. The triple interaction treatments (D1M1C2, D2M1C2) was characterized by recording it the highest percentage of Stevoside which amounted of (0.240%, 0.223%) compared to the D2M0C0 interaction treatment which recorded a significant decrease in the percentage of the same compound which reached of (0.030%) as shown in Table (13).

Table 13: Effect of planting dates, Mycorrhiza and bio-stimulators in the total percentage of Stevioside in dried roots of S. rebaudianaBertoni. plant.

Planting dates	Mycorrhiza vaccine	Bio-stimulators					Interaction D x M
		C0	C1	C2	C3	C4	
D1	M0	0.033	0.067	0.078	0.087	0.097	0.072
	M1	0.057	0.077	0.240	0.110	0.167	0.130
D2	M0	0.030	0.063	0.063	0.083	0.087	0.065
	M1	0.047	0.073	0.223	0.100	0.157	0.120
							Effect of planting dates D
Interaction (Planting dates x Bio-stimulators) D x C	D1	0.045	0.072	0.159	0.099	0.132	0.101
	D2	0.039	0.068	0.143	0.092	0.127	0.094
							Effect of fungal Mycorrhiza vaccine M
Interaction (fungal Mycorrhiza vaccine x Bio-stimulators) M x C	M0	0.032	0.065	0.071	0.085	0.092	0.069
	M1	0.052	0.075	0.232	0.105	0.162	0.125
Effect of Bio-stimulators C		0.042	0.070	0.152	0.095	0.127	
L.S.D 0.05							
D×M×C	M×C	D×C	D × M	C	M		D
0.0800	0.0600	0.0600	0.0500	0.0446	0.0298		N.S

Estimation the percentage of Rebaudioside A in dried roots of plant (%):

Table (14) shows a significant effect of most of the study factors individually when estimating the percentage of Rebaudioside A in the dried roots of the plant under study.The treatment (M1, C2, C4) was excelled by recording it the highest percentage of Rebaudioside A amounted of (0.065%, 0.074%, 0.071%) respectively, compared to M0 and C0 treatments, which recorded the lowest percentage of the same compound (0.041%, 0.029%), respectively, as shown in Table (14).While no significant differences were observed between the planting dates as shown in Table (14). The Bi-interaction treatments between planting dates and fungal Mycorrhiza vaccine (D × M), planting dates and bio-stimulators (D × C), and fungal Mycorrhiza vaccine and bio-stimulators (M × C) were significantly affected in the percentage of Rebaudioside A in dried roots of S .rebaudianaBertoni. plant.The bi-interaction treatments (D1M1, D1C2, M1C2, M1C4) were excelled by giving it the highest percentage of Rebaudioside A which amounted of (0.071%, 0.085%, 0.107%, 0.088%), respectively. while D1M0, D2M0, D2C0 and M0C0 treatments recorded the lowest percentage of Rebaudioside A which amounted (046%, 0.040%, 0.022%, 0.022%), respectively, as shown in Table (14).The triple interaction treatment (D × M × C) was significantly differed between study factors in the percentage of Rebaudioside A in the dried roots of S.rebaudianaBertoni. plant. The triple interaction treatment (D1M1C2) was characterized by

recording it the highest percentage of Rebaudioside A (0.127%) compared to the triple interaction treatment (D2M0C0) which recorded a significant decrease in the percentage of the same compound which reached of (0.010%) as shown in Table (14).

Table 14: Effect of planting dates, Mycorrhiza and bio-stimulators in the percentage of Rebaudioside A in the dried roots of S. rebaudianaBertoni. plant.

Planting dates	Mycorrhiza vaccine	Bio-stimulators					Interaction D x M
		C0	C1	C2	C3	C4	
D1	M0	0.033	0.040	0.042	0.053	0.060	0.046
	M1	0.037	0.043	0.127	0.060	0.093	0.071
D2	M0	0.010	0.037	0.040	0.043	0.047	0.040
	M1	0.033	0.040	0.087	0.050	0.083	0.059
							Effect of planting dates D
Interaction (Planting dates x Bio-stimulators) D x C	D1	0.035	0.042	0.085	0.057	0.077	0.059
	D2	0.022	0.039	0.064	0.047	0.065	0.047
							Effect of fungal Mycorrhiza vaccine M
Interaction (fungal Mycorrhiza vaccine x Bio-stimulators) M x C	M0	0.022	0.039	0.041	0.048	0.054	0.041
	M1	0.035	0.042	0.107	0.055	0.088	0.065
Effect of Bio-stimulators C		0.029	0.041	0.074	0.052	0.071	
L.S.D 0.05							
D×M×C	M×C	D×C	D × M	C	M		D
0.0368	0.0260	0.0260	0.0191	0.0184	0.0128		N.S

Total percentage of Steviol glycosides in dried roots of plant (%):

Table (12) shows that there is a significant effect of most of the study factors individually in the total percentage of Steviol glycosides in the dried roots of the S.rebaudianaBertoni. plant under study.The treatments (M1, C2) recorded the highest total percentage of Steviol glycosides in the dried roots of the plant which amounted of (0.191%, 0.266%), respectively. While the treatments (M0 and C0) recorded a significant decrease in the total percentage of Steviol glycosides in the dried roots of the plant, which amounted (0.110%, 0.070%), respectively. There were no significant differences between planting dates in the total percentage of Steviol glycosides in the dried roots of the S.rebaudianaBertoni. plant as shown in Table (15). The Bi-interaction treatments between planting dates and fungal Mycorrhiza vaccine (D × M), planting dates and bio-stimulators (D × C), and fungal Mycorrhiza vaccine and bio-stimulators (M × C) affected significant differences in the total percentage of Steviol glycosides in the dried roots of S.rebaudianaBertoni. plant. The bi-interaction treatments (D1C1, D1C2, M1C2, M1C4) were excelled by giving it the highest total percentage of Steviol glycosides of (0.202%, 0.244%, 0.339%, 0.250%), respectively, compared to the bi-interaction treatments (D2C0, D1C1, D2C1, D1C0, D2C0, M0C0) which recorded a significant decrease in the total percentage of Steviol glycosides, which amounted of (0.101%, 0.114%, 0.107%, 0.080%, 0.060%, 0.053%), respectively as shown in Table (15).The triple interaction treatment (D × M × C) was significantly differed between study factors in the total percentage of Steviol glycosides in the dried roots of S.rebaudianaBertoni. plant.The triple interaction treatments (D1M1C2) was characterized by recording it the highest total percentage of Steviol glycosides which amounted of (0.367%), compared to the D2M1C1, D1M0C1, D2M0C1, D1M1C0, D2M1C0, D1M0C0 and D2M0C0 treatments which showed a significant decrease in total percentage of Steviol glycosides (0.113%, 0.107%, 0.103 %, 0.100%, 0.094%, 0.080%, 0.066%, 0.040%), respectively as shown in Table (15).

Table 15: Effect of planting dates, Mycorrhiza and bio-stimulators in the total percentage of Steviol glycosides in the dried roots of S. rebaudianaBertoni. plant.

Planting dates	Mycorrhiza vaccine	Bio-stimulators					Interaction D x M
		C0	C1	C2	C3	C4	
D1	M0	0.066	0.107	0.120	0.140	0.157	0.118
	M1	0.094	0.120	0.367	0.170	0.260	0.202
D2	M0	0.040	0.100	0.103	0.126	0.134	0.101

	M1	0.080	0.113	0.310	0.150	0.240	0.179
							Effect of planting dates D
Interaction (Planting dates x Bio-stimulators) D x C	D1	0.080	0.114	0.244	0.155	0.209	0.160
	D2	0.060	0.107	0.207	0.138	0.187	0.140
							Effect of fungal Mycorrhiza vaccine M
Interaction (fungal Mycorrhiza vaccine x Bio-stimulators) M x C	M0	0.053	0.104	0.112	0.133	0.146	0.110
	M1	0.087	0.117	0.339	0.160	0.250	0.191
Effect of Bio-stimulators C		0.070	0.111	0.226	0.147	0.198	
L.S.D 0.05							
D×M×C	M×C	D×C	D × M	C	M	D	
0.1205	0.1069	0.1069	0.1004	0.0985	0.0796	N.S	

In the above, we find that there are significant differences between the factors of this study were represented by the fungal Mycorrhiza vaccine (M) and bio-stimulators (C) in the estimation of medical compounds belonging to the steviol glycosides group in the different parts of Stevia rebaudianaBertoni. plant as shown in tables (8-15), while there were no significant differences between the planting dates (D) of the plant under study as shown in Tables (7-15). Stevioside and Rebaudioside A are considered a component of Steviol glycoside formed in plant tissues containing chlorophyll pigment. Chloroplast is considered the starting point for the synthesis of steviol glycosides (Pol et al., 2007).It is The initiator compounds that create in chloroplasts the important role in the formation of the glycosides. Therefore, these glycosides are collected in the mature leaves more than the leaves newly grown, which makes them sweet taste.Mature leaves are characterized by the fact that most of the chlorophyll pigment has a role in the formation of the glycosides. On the other hand, nitrogen is considered an important element in the synthesis of proteins, enzymes, coenzymes, nucleic acids and phytochrome.Thus, chlorophyll has an important role in the biochemical processes required by Stevia to increase Stevioside and Rebaudioside A (Inugraha et al., 2014).The superiority of the M1 treatment was attributed by recording it the highest percentage of all medical compounds belonging to the steviol glycosides group in all dried plant parts (leaves, branches, stems, roots) estimated for the plant under study as shown in Tables (7-15).The treated roots of medicinal plants with the Mycorrhiza fungus improves the production of medicinal compounds that can make the plant more resistant and tolerant of vital and non-vital stresses as well as useful substances for human health (Gianinazzi et al., 2010). Steviol glycosides are made up of two Diterpenes with biochemical pathways that share four steps with the formation of the Gibberellic acid (Brandle and Telmer, 2007).Banchio et al. (2010) note that the synthesis of terpenoids is based on: primary metabolites, photosynthesis, pathways of carbon oxidation and processed energy. The microorganisms of all species are considered able to increase primary metabolite compounds by improving photosynthesis as well as their improvement of plant content from elements and growth. The effect of bio-fertilizers, including microorganisms as a biomass fertilizer in the production of sub-metabolites, to the events of the balance of nutrient materials and water in the root area of the plant.The reason for the significant increase of the Stevioside and Rebaudioside A group returning to the group of sub-metabolites as shown in Table (7-15) in the plant under study is due to the association of these compounds with the stimulators produced by the Mycorrhiza fungi, which may release the hormones and some highly efficient molecules as shown in Table (1), which may act as catalysts materials to induce the production of Stevioside and Rebaudioside A (Vafadar et al., 2014; Mandal et al., 2013).The addition of Mycorrhiza improves the production of secondary compounds in S.rebaudianaBertoni. plant. Especially the Stevioside compound and the Rebaudioside A compound. Mycorrhiza increases the susceptibility of the root system to absorption of water and nutrient elements such as nitrogen, phosphorus, potassium, magnesium, iron, copper, manganese and zinc, as well as an increase in chlorophyll pigment, total carbohydrates and jasmonique acid in the total vegetative. The increase in the concentration of total carbohydrates and jasmonique acid is associated with increased synthesis of Steviol glycosides (SGs) by the methyl erythritol phosphate pathway.The work of the Mycorrhiza as a mediator works to induce an increase in steviolic glycosides with the participation of each mechanisms associated by nutrient and non-nutrient (Mandal et al., 2013).From the above, the reason for recording M0 treatment a significant decrease in the percentage of Steviol glycosides in all dried plant parts of the S.rebaudianaBertoni plant. as shown in Tables (7-15). The reason for excelling C2 treatment by recording it the highest percentage in all medical compounds belonging to the Steviol glycosides group in all dried plant parts (leaves, branches, stems, roots) of the studied plant as shown in Tables (7-15) is due to the catalytic effect of chitosan resulting from its constituents as shown in Tables (4) in the traits of vegetative growth and

enhancement of photosynthesis, which leads to a transformation in the division of vegetative growth to the rest of the plant parts.Chitosan is characterized by its stimulating effect on the process of absorbing water and essential nutrient elements by controlling the Osmotic pressure of the cell and thus increasing the active enzymes. Moreover, Chitosan increases the activity of the main enzymes responsible for nitrogen metabolism such as nitrate reductase, glutamine synthetase, Protease as well as improved nitrogen transport (Gornik et al., 2008; Guan et al., 2009; Marschner, 2013).Nitrogen is an important element in the formation of the chlorophyll molecule responsible for the process of converting photovoltaic energy into chemical energy, which is one of the biosynthesis centers of Steviol glycosides (SGs).The reason for excelling C4 treatment by recording it the highest percentage of Rebaudioside A in dried leaves and roots as shown in Tables (8, 14), total Steviol glycosides (SGs) in dried leaves as shown in Table (9) and Stevioside compounds in dried branches and stems as shown in Table (10) is may be due to the fact that seaweed extracts increased nutrient absorption from the soil as a result of its constituents as shown in Table (6) as well as its improved photosynthesis and vegetative growth, affecting the improved vegetative growth traits and their content of elements and compounds active (Mooney and Van Staden, 1986).The reason for excelling of D1M1 treatment by recording it the highest percentage in all medical compounds belonging to the Steviol glycosides group in all dried plant parts (leaves, branches, stems, roots) which estimated in it for the plant under study as shown in Tables (7-15) is due to the effect of the first planting date D1 on the growth of the Mycorrhiza vaccine M1 resulting in an increase in the percentage of all medical compounds belonging to the Steviol glycosides group, and may be to the relationship of these medical compounds with stimulantors produced by the Mycorrhiza fungi, which may release hormones and Some bioactive molecules which produced by other microorganisms in the soil as shown in Table (1), which may act as catalysts that stimulate the production of Stevioside and Rebaudioside A, which are due to the Steviol glycosides group (Vafadar et al., 2014; Mandal et al., 2013).Since there were no significant differences between the planting dates (D) in the percentage of all medical compounds belonging to the Steviol glycosides group (Staphoside, Rebaudioside A, and the total localities of the steviol classics) in all plant parts of the plant under study as shown in Table (7-15) and the treated of root system fungal Mycorrhiza vaccine, which led to increased induction of formation medical compounds by the microorganisms during the second planting date D2. This increase was significant in the Stevioside compound in the bi-interaction treatment (D2M1) in the dried leaves of the plant under the study as shown in Table (7,13), as well as in the rest of the compounds and different parts of the plant, there was an increase in the same bi-interaction treatment (D2M1), but they were not significant as shown in Table (7-15).This may be due to the fact that the Stevioside compound has been induced by the addition of Mycorrhiza to the root of the plant, resulting in an increase in its percentage in the leafy part of the plant because the green plastids in the leaf are considered the synthesis regions of this compound (Stevioside).Chitosan stimulates the process of absorbing water and essential nutrient elements by controlling the Osmotic pressure of the cell and thus increasing the active enzymes, as well as increasing the activity of the main enzymes responsible for nitrogen metabolism such as nitrate reductase, glutamine synthetase, Protease and improves the nitrogen transport (Gornik et al. , 2008; Guan et al., 2009).Nitrogen is an important element in the formation of the chlorophyll molecule responsible for the process of converting photovoltaic energy into chemical energy, which is one of the biosynthesis centers of Steviol glycosides (SGs).Bi-interaction treatment (D1C4) was characterized by recording it the highest percentage in all medical compounds belonging to the stevioside group, Rebaudioside A, and the total of Steviol glycosides (SGs)in the dried leaves of the plant under study as shown in Tables (7-15).This is due to the interaction between the environmental conditions that characterized the first planting date D1 as shown Table (2) with the spraying process of the total vegetative with seaweed extract C4 and what the advantage of this extract from the components as shown in Table (6) which worked together on the events of an increase in the photosynthesis process in the leaves and since the leaf is the main center for the presence of green plastids, which are considered the synthesis centers of Steviol glycosides, which led to an increase in the percentage of those medical compounds.The reason for excelling D2C2 and D2C4 treatments by giving it the highest percentage of Rebaudioside A and total SGs in the dried leaves of the plant under study as shown in Table (8, 9) is due to the absence of significant differences between planting dates (D) in the percentage of all medical compounds belonging to the stevioside group (stevioside and Rebaudioside A and the total Steviol glycosides (SGs) in all plant parts under study as shown in Table (7-15), resulting in the emergence of catalytic effect for each treatment with Chitosan (C2) and treatment with seaweed extract (C4) for photosynthesis at the second planting date, which resulted in an increase in photosynthesis due to increased concentration of chlorophyll by the components of both Chitosan and seaweed extract as shown in Tables (4, 6), which positively reflected the increase in the percentage of each Rebaudioside A compound and the total Steviol glycosides (SGs) in the dried leaves of the plant under study.the reason for excelling D1C3 treatment by recording it the highest percentage of Rebaudioside A compound in dried leaves as shown in

Table (8) is due to the combined effect of the environmental conditions for the first planting date D1 as shown in Table (2) and The spraying treatment with dry bread yeast extract (C3) and its components as shown in Table (5) resulted in increased photosynthesis, which resulted in an increase in the concentration of chlorophyll in the leaves. Which may be due to its possibly increasing the percentage of Rebaudioside A compound in the dried leaves of the plant under study.The reason for excelling the interaction treatment (M1C2) by recording it the highest percentage of all medical compounds belonging to the Steviol glycosides group for all the plant parts under study as shown in Table (7-15) may be due to there was a synergistic relationship between the spraying treatment with cetosan C2 and the addition of the fungal Mycorrhiza vaccine M1 to the root system and the content of the sap plant from nutrient elements (Wang et al., 2007), which stimulated the photosynthesis process and thus increase the percentage of secondary metabolites (Steviol glycosides SGs), Chitosan worked on the one hand, on the improvement of biomass and enhanced the activity of key enzymes as well as it is a vital catalyst for secondary metabolites such as Rebaudiosid A compound (Mehregan et al., 2017).On the other hand, the addition of Mycorrhiza improved the production of secondary compounds in S.rebaudianaBertoni. plant, Especially the Stevioside compound and the Rebaudioside A compound.The reason for the Mycorrhiza work to increase the size of the root system, which positively reflects the increase in the biomass of the total vegetative. Chitosan has a catalytic effect on the process of absorbing water and essential nutrient elements by controlling the osmotic pressure of the cell and thus increasing the active enzymes. Moreover, Chitosan increases the activity of the main enzymes responsible for Nitrogen metabolism, such as nitrate reductase, glutamine synthetase, Protease, As well as improved nitrogen transport (Gornik et al., 2008; Guan et al., 2009). As well as an increase in chlorophyll, total carbohydrate and jasmonique acid in the total vegetative.The increase in the concentration of both total carbohydrates and jasmonique acid is associated with an increase in the synthesis of Steviol glycosides (SGs) by the Methyl erythritol phosphate pathway and concludes that the microorganism acts as an intermediary to increase the Steviol glycosides with the participation of each mechanisms associated by nutrient and non-nutrient (Mandal et al., 2013).The reason for excelling treatment of M1C4 by recording it the highest percentage of Rebaudioside A compound and the total Steviol glycosides in the dried roots of the plant under study as shown in Table (14,15) is due to the seaweed extract mechanism by influencing the physiological work of macro and micronutrients, amino acids and vitamins, It is preferable to contain seaweed extract on plant growth regulators such as Gibberellins, Auxins (IAA) and cytokinines, which in turn affect cellular metabolism in treated plants, Leading to an increase in vegetative and root growth and crop productivity (Ördög et al., 2004).Thus improving the photosynthesis process of and thus vegetative growth, which affected the vegetative content of the active elements and compounds (Mooney and Van Staden, 1986), and the use of seaweed extract has stimulated the growth of the Hypha of Mycorrhiza. The absorption of nutrients from the soil and their conversion through these Hypha from the root to the total vegetative which increased the growth through the effect of fungal vaccine to increase the plant root area and thus facilitate the penetration of soil as well as increase the activity of secretions enzymatic of the Mycorrhiza fungus existence in the root or through its Hypha. The Mycorrhiza fungus improves the growth of the plant through the release of Hypha fungal from growth regulating substances such as Gibberellins and Auxins, as well as seaweed extract from hormones such as Auxins, Gibberellins and cytokinines (Smith, Red, 1997; Barker, Taqu, 2000; Jensen, 2004).Evidence suggests that the addition of the Mycorrhiza fungus (As a fungal bio-fertilizer) to plant roots led to changes in primary metabolism including photosynthesis, water absorption and drought tolerance (Ruiz-Lozano, 2003) as well as changes in secondary metabolites Such as changes in the dynamics of plant hormones, structural alterations and the activation of defense mechanisms (Allen et al., 1982), which may have an effect on the significant increase of Rebaudioside A in the roots of dried plants in this study which is a secondary metabolite due to the relationship of this compound with the stimuli produced by Mycorrhiza fungus Which may have released the hormones and some of the bioactive molecules produced by other microorganisms in the soil that act as catalysts that induce the synthesis of Rebaudioside A.From the above factors, from the single effect of each of the study factors and of the binary interference factors of the study factors, we can understand why D1M1C2 is excelled by recording it the highest percentage of all medical compounds belonging to the steviol glycosides group (Staphoside, Rebaudioside A), and the total local steviol glycosides SGs in all dried plant parts (leaves, branches, stems, roots) that were estimated for the plant under study as shown in Table (7-15).Due to the absence of significant differences between planting dates, we note the superiority of the triple interaction treatment (D2M1C2) by recording it the highest percentage in the total localities of the steviol glycosides in each of the leaves dried and the percentage of the steviol glycosides in the dried roots as shown in Table (9, 14).The increase in the percentage of steviol glycosides in the roots may be due to the fact that the root content of these compounds increases 7 times after the transition to plant propagation phase in S. rebaudiana Bertoni. plant where increasing from 0.05% to 0.35% (Genus, 2003).The

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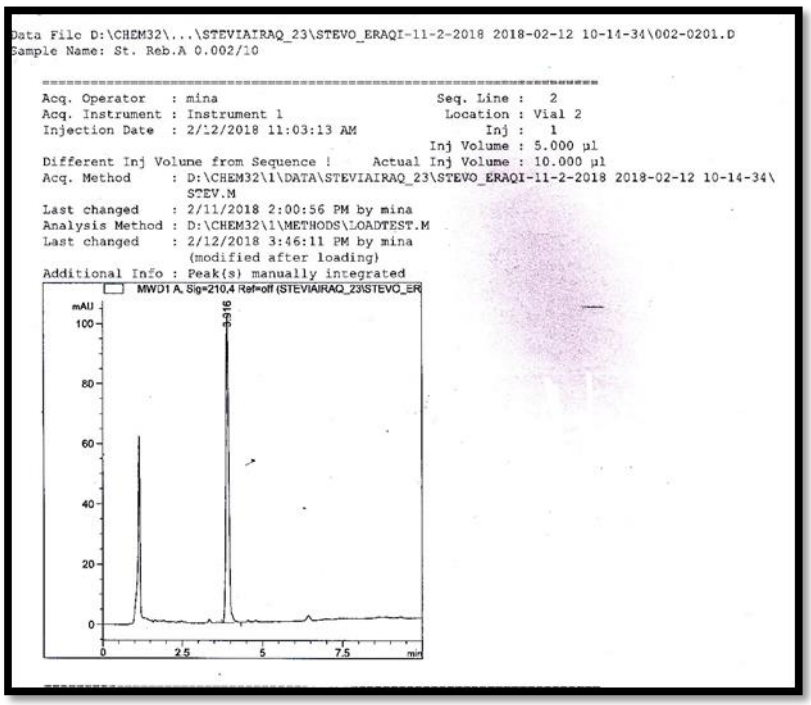
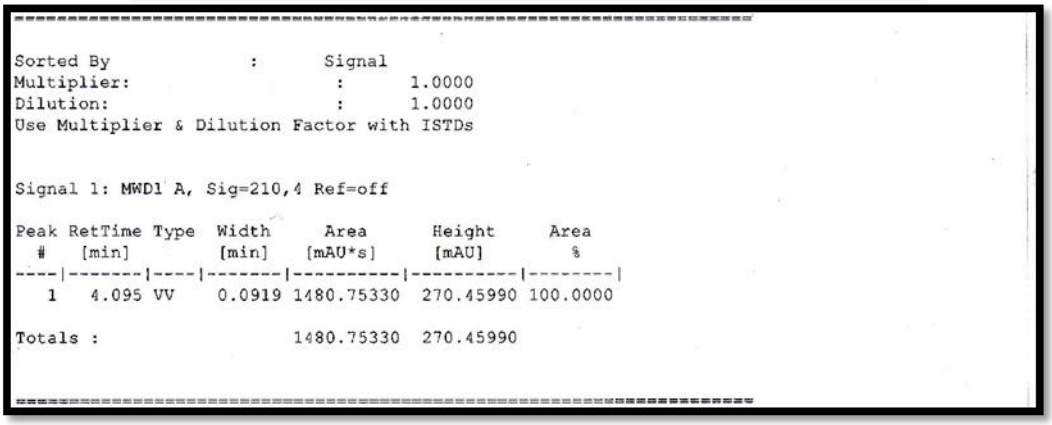
plant under study was as seedlings that were grown in the laboratory and then adapted in the lath house and then planted in the land of the plastic house as well as the effect of the study treatments, which led to the occurrence of this increase.





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Dilution:                    :      1.0000
Use Multiplier & Dilution Factor with ISTDs


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Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]    [mAU*s]    [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1   4.095 VV    0.0919 1480.75330  270.45990 100.0000

Totals :                  1480.75330  270.45990
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Figure 1: Standard curve, retention time, peak area and concentration of Stevioside compound

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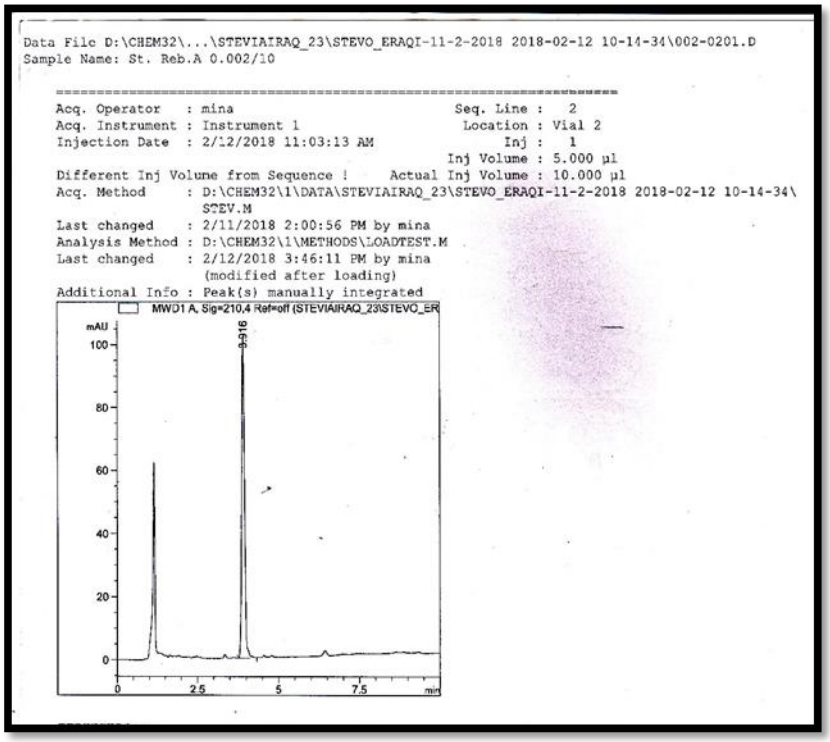

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Multiplier: : 1.0000
Dilution: : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: MWD1 A, Sig=210,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	3.916	VB	0.0927	596.54303	101.24886	100.0000
Totals :				596.54303	101.24886	

Figure 2: Standard curve, retention time, peak area and concentration of Rebaudioside A compound.

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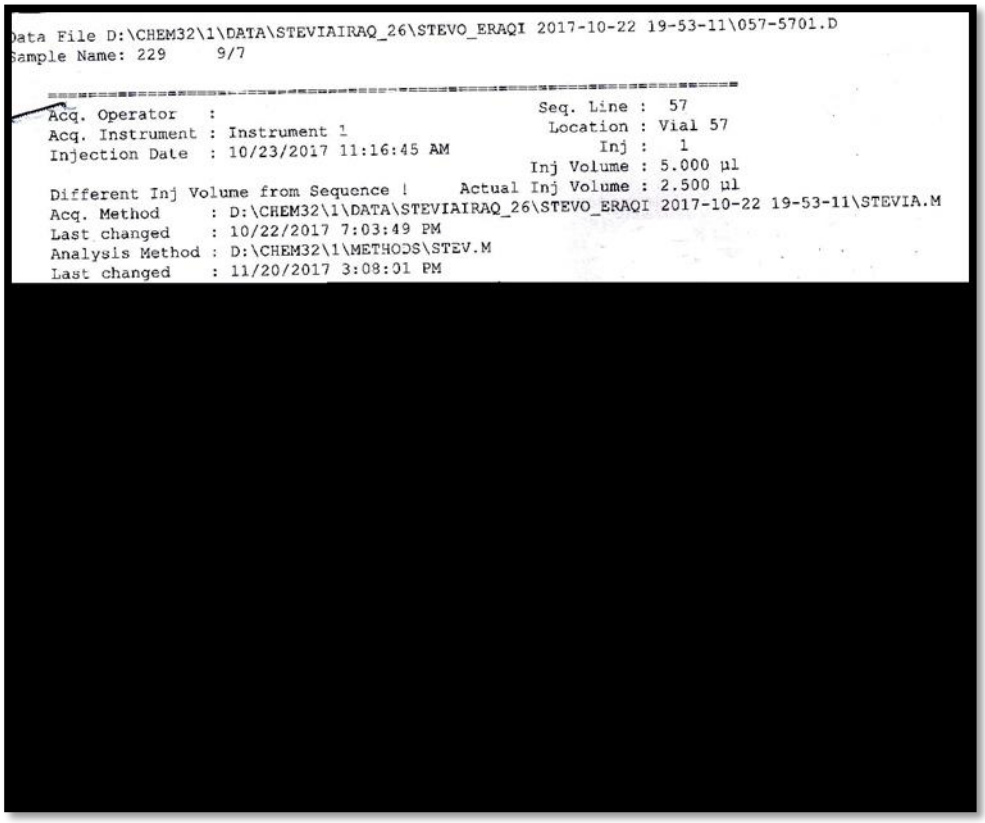


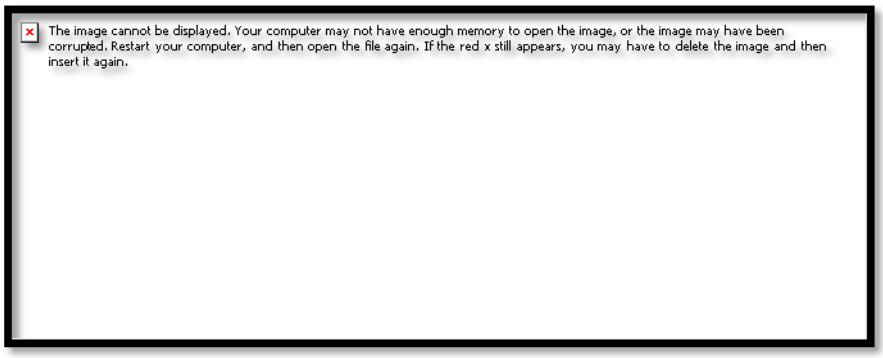
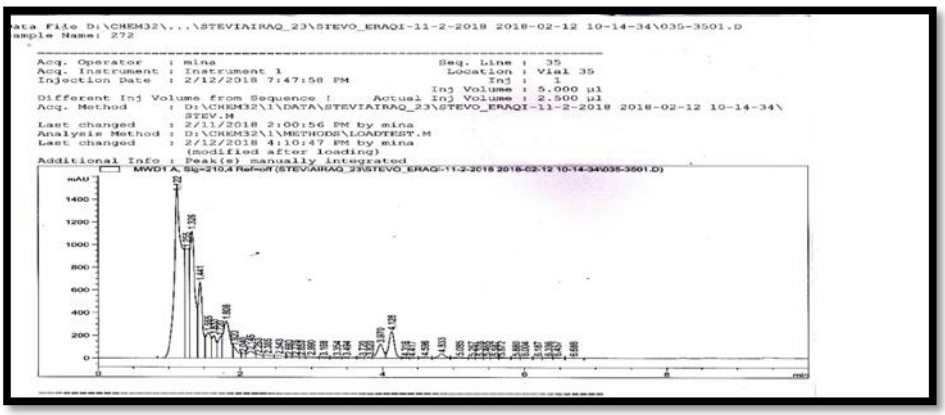


Figure 3: Results of analysis of high performance liquid chromatography (HPLC) for Stevioside and Rebaudioside A compounds and some other medical compounds found in the dried leaves sample of S.rebaudianaBertoni plant for one of the replicates of excelled triple interaction treatment (D1M1C2).



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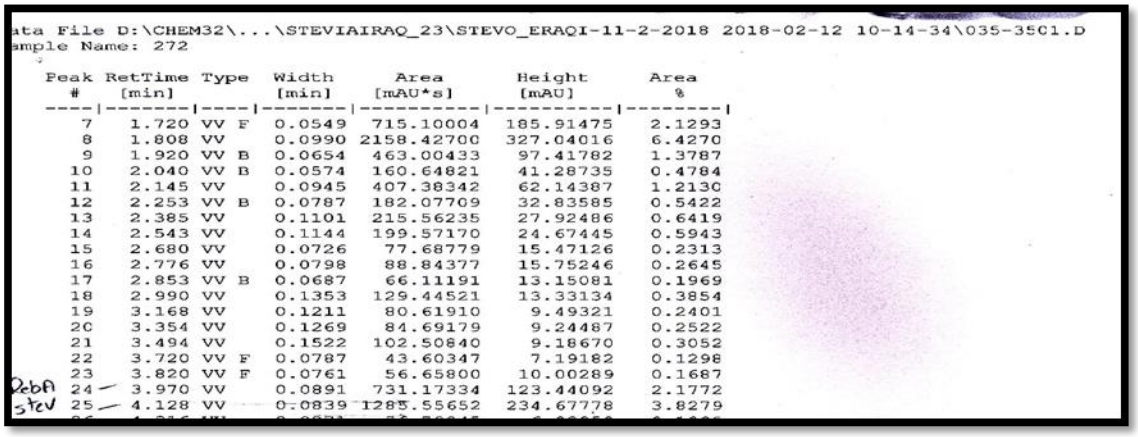

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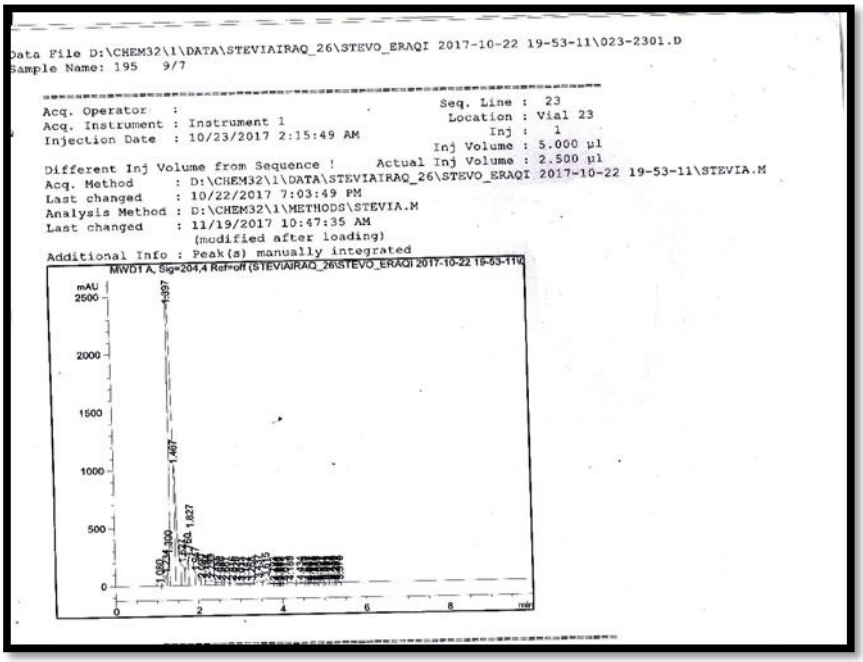
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
7	1.720	VV F	0.0549	715.10004	185.91475	2.1293
8	1.808	VV	0.0990	2158.42700	327.04016	6.4270
9	1.920	VV B	0.0654	463.00433	97.41782	1.3787
10	2.040	VV B	0.0574	160.64821	41.28735	0.4784
11	2.145	VV	0.0945	407.38342	62.14387	1.2130
12	2.253	VV B	0.0787	182.07709	32.83585	0.5422
13	2.385	VV	0.1101	215.56235	27.92486	0.6419
14	2.543	VV	0.1144	199.57170	24.67445	0.5943
15	2.680	VV	0.0726	77.68779	15.47126	0.2313
16	2.776	VV	0.0798	88.84377	15.75246	0.2645
17	2.853	VV B	0.0687	66.11191	13.15081	0.1969
18	2.990	VV	0.1353	129.44521	13.33134	0.3854
19	3.168	VV	0.1211	80.61910	9.49321	0.2401
20	3.354	VV	0.1269	84.69179	9.24487	0.2522
21	3.494	VV	0.1522	102.50840	9.18670	0.3052
22	3.720	VV F	0.0787	43.60347	7.19182	0.1298
23	3.820	VV F	0.0761	56.65800	10.00289	0.1687
24	3.970	VV	0.0891	731.17334	123.44092	2.1772
25	4.128	VV	0.0839	1285.55652	234.67778	3.8279

Reb A
Stev

Figure 4: Results of analysis of high performance liquid chromatography (HPLC) for Stevioside and Rebaudioside A compounds and some other medical compounds found in the dried branches and stems sample of S.rebaudianaBertoni plant for one of the replicates of excelled triple interaction treatment (D1M1C2).



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Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
9	1.947	VV B	0.0791	593.82843	103.30325	2.2471
10	2.090	VV	0.0777	213.11601	39.03864	0.8065
11	2.194	VV	0.0653	159.65382	34.92794	0.6042
12	2.299	VV	0.1180	351.26605	41.80162	1.3292
13	2.455	VV	0.1214	179.04147	21.89639	0.6775
14	2.560	VV B	0.0538	58.16458	18.02293	0.2201
15	2.667	VV	0.1011	128.28357	18.00647	0.4854
16	2.816	VV	0.1001	112.54037	15.98571	0.4259
17	2.920	VV B	0.0954	95.12643	13.98449	0.3600
18	3.033	VV B	0.0739	57.48938	10.50325	0.2175
19	3.142	VV	0.0959	69.58659	10.15495	0.2633
20	3.268	VV	0.0916	62.52037	9.65000	0.2366
21	3.437	VV	0.1182	221.66502	27.46291	0.8388
22	3.615	VV	0.1141	326.31387	42.26160	1.2348

Reb A
Stev

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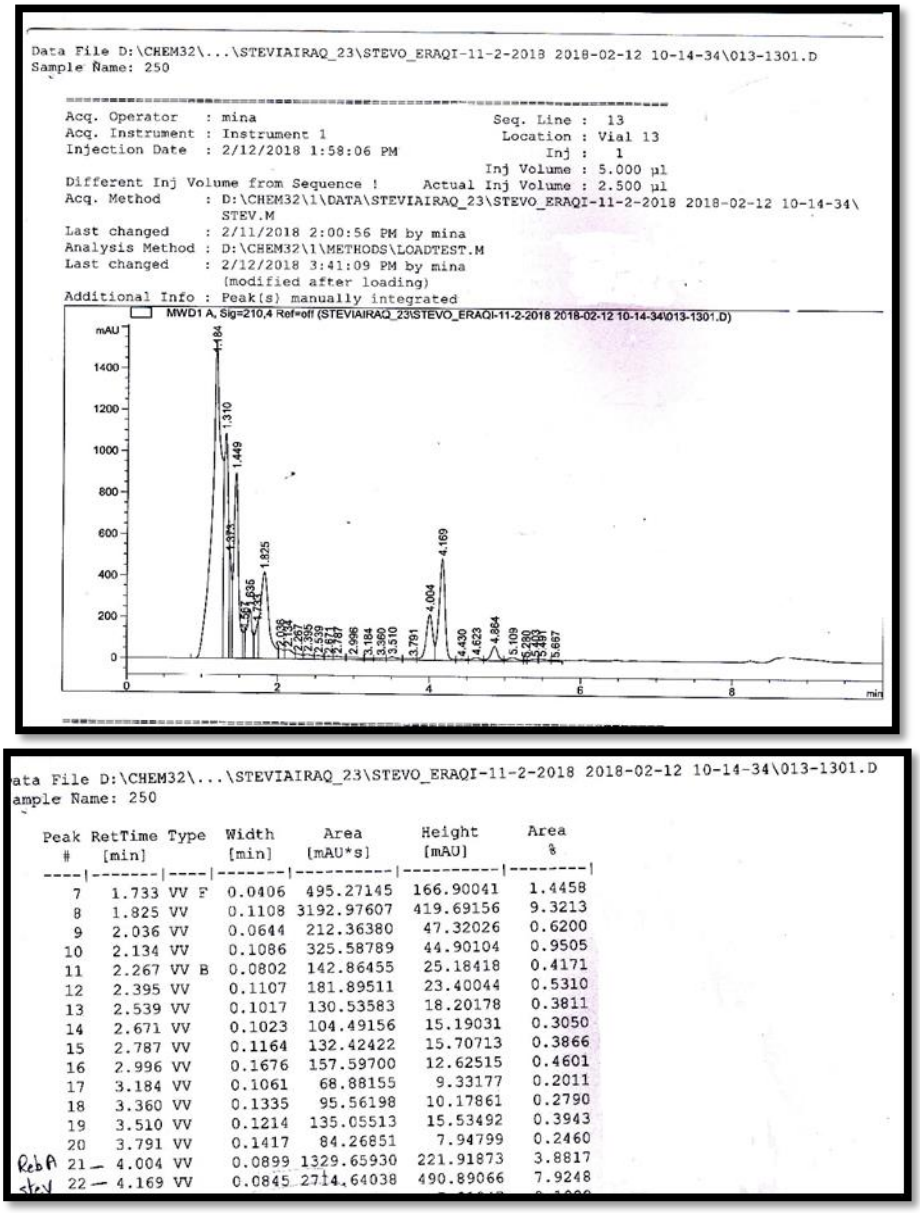
Figure 5: Results of analysis of high performance liquid chromatography (HPLC) for Stevioside and Rebaudioside A compounds and some other medical compounds found in the dried roots sample of S.rebaudianaBertoni plant for one of the replicates of excelled triple interaction treatment (D1M1C2).



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Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
7	1.733	VV F	0.0406	495.27145	166.90041	1.4458
8	1.825	VV	0.1108	3192.97607	419.69156	9.3213
9	2.036	VV	0.0644	212.36380	47.32026	0.6200
10	2.134	VV	0.1086	325.58789	44.90104	0.9505
11	2.267	VV B	0.0802	142.86455	25.18418	0.4171
12	2.395	VV	0.1107	181.89511	23.40044	0.5310
13	2.539	VV	0.1017	130.53583	18.20178	0.3811
14	2.671	VV	0.1023	104.49156	15.19031	0.3050
15	2.787	VV	0.1164	132.42422	15.70713	0.3866
16	2.996	VV	0.1676	157.59700	12.62515	0.4601
17	3.184	VV	0.1061	68.88155	9.33177	0.2011
18	3.360	VV	0.1335	95.56198	10.17861	0.2790
19	3.510	VV	0.1214	135.05513	15.53492	0.3943
20	3.791	VV	0.1417	84.26851	7.94799	0.2460
RebA std 21	4.004	VV	0.0899	1329.65930	221.91873	3.8817
22	4.169	VV	0.0845	2714.64038	490.89066	7.9248

Figure 6: Results of analysis of high performance liquid chromatography (HPLC) for Stevioside and Rebaudioside A compounds and some other medical compounds found in the dried leaves sample of S.rebaudianaBertoni plant for one of the replicates of excelled triple interaction treatment (D2M0C0).

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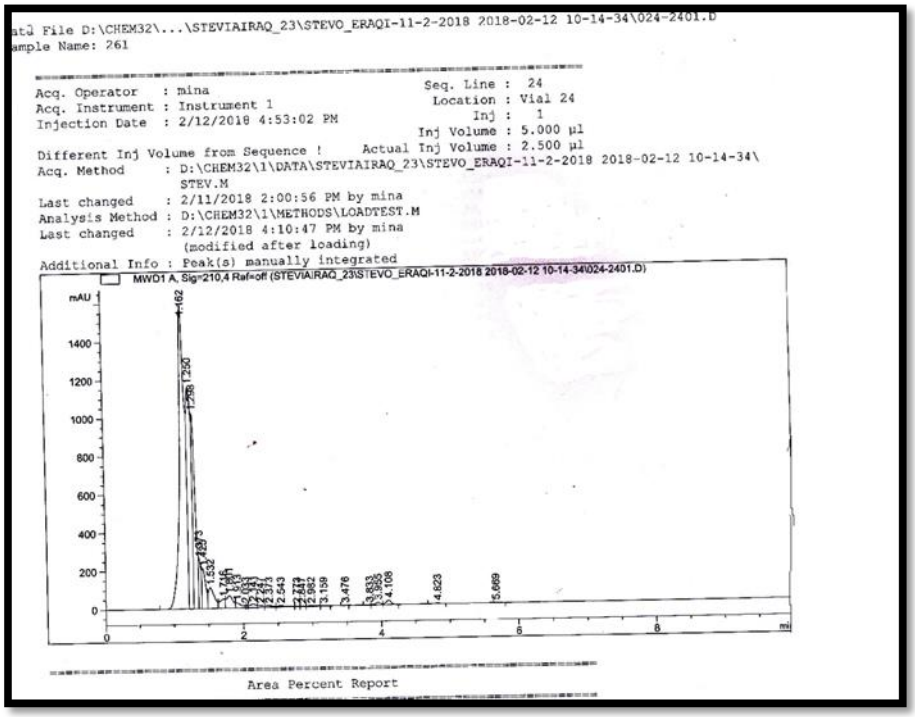

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Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
7	1.716	VV	0.0761	252.26266	47.37552	1.2172
8	1.801	VV	0.0951	415.15411	62.81157	2.0032
9	1.913	VV B	0.0818	149.10577	24.92806	0.7195
10	2.033	VV B	0.0321	20.19436	10.49822	0.0974
11	2.141	VV	0.0959	114.53098	17.15015	0.5526
12	2.247	VV B	0.0777	53.00392	9.13863	0.2558
13	2.373	VV	0.1177	63.18359	7.70030	0.3049
14	2.543	VV	0.1522	76.11924	6.71729	0.3673
15	2.773	VV B	0.0673	16.30632	3.44131	0.0787
16	2.847	VV B	0.0738	15.81223	3.19300	0.0763
17	2.982	VV	0.1365	31.63111	3.22225	0.1526
18	3.159	VV	0.1130	14.89981	1.86821	0.0719
19	3.476	VV	0.0946	11.21335	1.75430	0.0541
20	3.833	VV	0.0742	10.49648	2.10916	0.0506
Reb A 21 →	3.955	VV	0.0914	78.31612	13.16246	0.3779
Stev 22 →	4.108	VV	0.0845	129.60960	23.42572	0.6254

Figure 7: Results of analysis of high performance liquid chromatography (HPLC) for Stevioside and Rebaudioside A compounds and some other medical compounds found in the dried stems and branches sample of S.rebaudianaBertoni plant for one of the replicates of excelled triple interaction treatment (D2M0C0).

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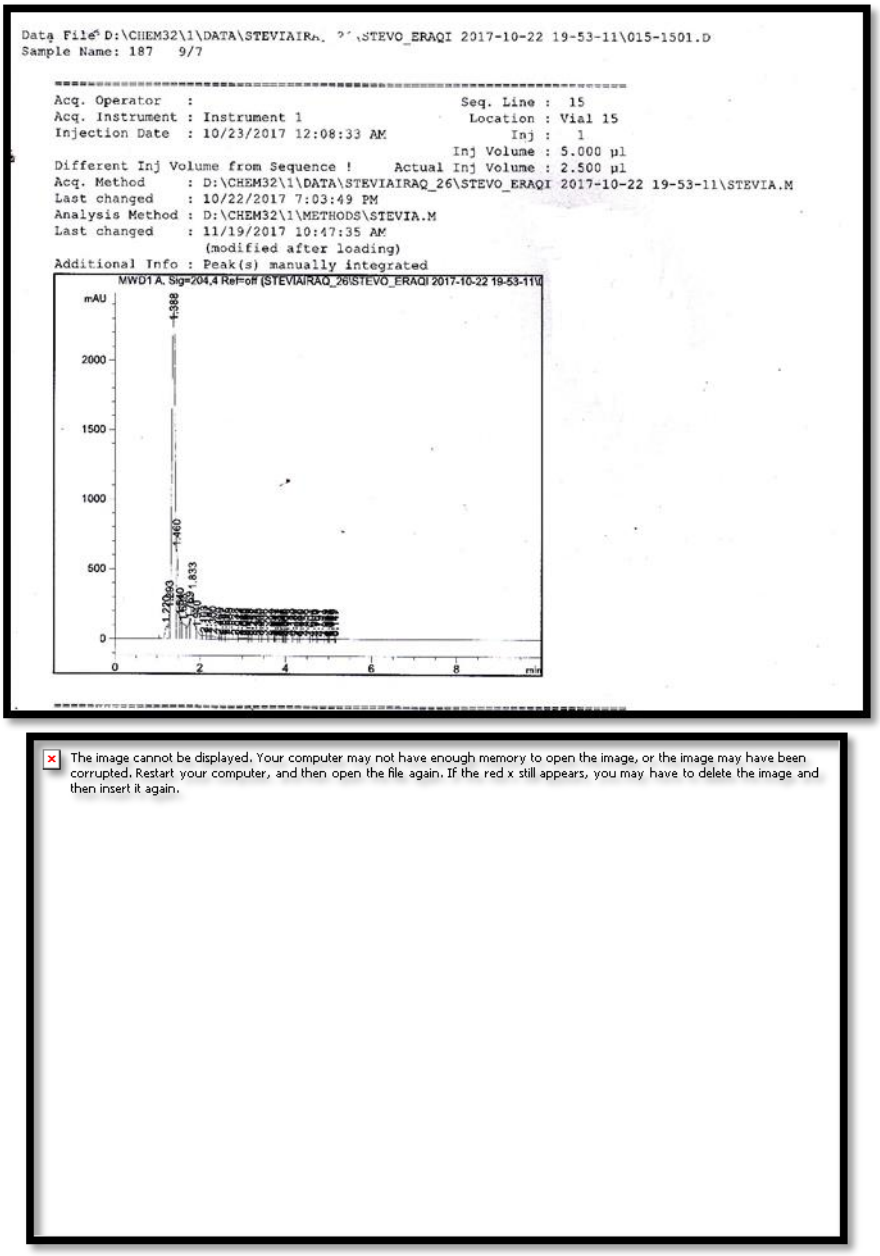

Figure 8: Results of analysis of high performance liquid chromatography (HPLC) for Stevioside and Rebaudioside A compounds and some other medical compounds found in the dried roots sample of S.rebaudianaBertoni plant for one of the replicates of excelled triple interaction treatment (D2M0C0).

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