Plant Archives Vol. 19 No. 1, 2019 pp. 1113-1129 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

EFFECT OF PLANTING DATE, ADDING OF MYCORRHIZA, BIO-STIMULATORS AND INTERACTIONS AMONG THEM FOR IMPACT ON SOME VEGETATIVE GROWTH CHARACTERS OF THE *STEVIA REBAUDIANA* BERTONI CULTIVATED IN IRAQ

Ziena M. Abdul-Qader* and Kareem M. Rabie

Department of Horticulture and Gardening Landscape, College of Agricultural Engineering Sciences, University of Baghdad, Iraq

Abstract

This experiment was conducted in one of the plastic house in the research station B belonging to 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 obtained in this study showed that the first planting date D1 is excelled on the second planting date D2 in all vegetative growth characters and yield dried leaves. While the treatment of the addition of the fungal Mycorrhiza vaccine (M1) to the root system led to significant increase in the vegetative growth compared to the control treatment (M0).The spraying treatment for leaves with seaweed extract (C4) showed a significant increase in most vegetative growth characters compared with all treatments, While the spraying treatment for leaves with Chitosan was significantly excelled in the leaves content of a,b and total chlorophyll. Triple interaction treatment (D1M1C4) showed significant superiority in most vegetative traits, including: plant height, fresh and dry weight of leaves, number of total leaves for plant, plastic house yield of dried leaves, total leaf area (101.67 cm, 109.67g.$plant^{-1}$, 53.20 g.$plant^{-1}$, 216.67 leaf.$plant^{-1}$, 31.92 kg/plastic house, 29.77 dm^2), respectively, while the triple interaction treatment (D1M1C2) recorded significant excelling on the rest of the treatments in both the leaves content of a,b and total chlorophyll (4.58, 2.29, 6.88 mg.100 g^{-1} fresh weight), respectively.

Key words : Planting date, mycorrhiza, chitosan, seaweed extract and yeast extract.

Introduction

Stevia rebaudiana Bertoni plant is considered one of the important medicinal plants belonging to the Asteraceae family (Mubarak *et al*., 2008). Scientific sources indicate that the native habitat of *Stevia rebaudiana* Bertoni is South America, specifically east of Paraguay and Brazil (Ahmed *et al*., 2011). It is widely used commercially in the food and pharmaceutical industries. The medicinal significance of this plant is due to high content for its leaves from 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 and Rebaudioside A compound (Varanuj and Chatchai, 2009; Ahmed *et al*., 2011; Farooqi and Sreeramu, 2004). The medical significance of Stevioside and Rebaudioside A is due to the many pharmaceutical and therapeutic properties they possess (Goyal and Samsher, 2010; Pemba and Sharangi,

Author for correspondence : E-mail : ziena73mohammed@gmail.com

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. Planting date is considered a one of the factors determining the growth of *Stevia rebaudiana* Bertoni plant and its development in the presence of the optimal environmental conditions during the period of plant growth, which are positively reflected in obtaining the highest rate of growth of the plant and its yield of leaves (Maheshwar, 2005). The date of transfer of plants to the permanent field plays a significant role in determining the quantitative and qualitative traits and vegetative growth characters for medicinal plants (Khan *et al*., 2012) because of the prevailing environmental conditions which positively or negatively reflect on vegetative growth characters and formation of active compounds (Maheshwar, 2005; Khan *et al*., 2012; Talei *et al*., 2012). Beement (2012) found that the best planting date for cultivating of *S. rebaudiana* Bertoni plant in Ethiopia, which their temperatures ranging from 6-25°C during the seedling stage after transfer to the permanent field, although the plant can grow during its advanced stages of vegetative growth (6-25°C) in the same country. The reason for this was that the temperature of 6-25°C was optimal for the beginning of seedling growth in the field, which was positively reflected in the increase in vegetative growth indicators (plant height, fresh and dry weight of leaves, number of leaves, stem diameter, concentration of total chlorophyll) and thus increase the dry yield of leaves. Several studies have found that the inoculating root of many medicinal plants with mycorrhiza work to improve the growth of these plants, biomass and yield by adding nutrients for plant and producing growth regulators as well as improving soil fertility by adding and synthesizing nutrients in the soil where the host plant grows (Hoseini *et al*., 2015; Earanna, 2007; Hemavathi *et al*., 2006). The reason of this attributed as a result of what Mycorrhiza fungus is causing the colonization of the root zone for the plant, resulting in a marked balance between the root system and total vegetative due to an increase in the absorption of water and nutrient elements necessary for growth, which is positively reflected in improving growth and increasing biomass (Harrier and Sawczak, 2000; Singh *et al*., 2000; Rai *et al*., 2001; Varma *et al*., 2001; Kumari *et al*., 2003; Waller *et al*., 2005). Wu and Xin (2006) found that the Mycorrhiza fungus vaccine worked on an increase in the rate of photosynthesis in plants that their roots treated with it. As well as the treatment of seedlings Medicinal plants with Mycorrhiza fungus works to increase the content of fresh leaves from chlorophyll (Kaschuk *et al*., 2009; Vafadar *et al*., 2014) as the work of the Mycorrhiza fungus to produce substances such as hormones stimulate the production of active compounds to stimulate the production of Stevioside and Rebaudioside A (Mandal *et al*., 2013; VAfadar *et al*., 2014). Plant bio-stimulators are divided into two forms: either naturally created such as amino acids, yeast extract, seaweed extract, Chitosan, hormones and plant growth regulators or artificially synthesized such as synthetic hormones, phenolic compounds, inorganic salts and essential elements (Rafiee *et al*., 2016). Several studies have indicated that spraying the total vegetation of plant with seaweed extract is of great benefit to the plant by giving a significant increase in vegetative growth indices (Saif-Eldeen *et al*., 2014; Fayza *et al*., 2015; Salama *et al*., 2016). The reason for this is attributed to the content of the seaweed extract on micro and macro mineral elements, hormone-like substances such as Sterols, Polyamines, Cytokinines, Gibberellin, Auxins and Abscisic Acid as well as containing anti-stress and anti-oxidative substances and internal gene organizations that Self-response to stress (Craigie, 2011; Calvo *et al*., 2014). The use of foliar spraying for plants with chitosan increases the photosynthesis process, which reflects positively on the occurrence of an increase in vegetative growth indicators instead of stimulating the growth and activity of some beneficial microorganisms found in soil such as Mycorrhiza (Murphy *et al*., 2000; Kim *et al*., 2005; Lowe *et al*., 2012). The spraying of leaves with chitosan improves the transmission of nitrogen, which is an important element in the formation of the chlorophyll molecule, Which is considered one of the bio-synthesis centers of Steviol glycosides (SGs) (Górnik *et al*., 2008; Guan *et al*., 2009). Due to the absence of previous studies on the cultivation of *Stevia rebaudiana* Bertoni. plant in Iraq and the importance of medicinal plants and the important role of these factors in impact of vegetative growth, this study aims to demonstrate the effect of the planting date and Mycorrhiza and bio-stimulators and the interaction between them in vegetative growth indicators and yield from dried leaves of *Stevia rebaudiana* Berton plant cultivated in Iraq.

Materials and Methods

The experiment was conducted in one of the plastic house in the research station B belonging to 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 rows and 1 m between the experimental units within the single raw. Nine samples were taken from 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.

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 (*Glomus mossea*) 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 Abdel-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 (Joud smartfert) is given with a fertilizer combination (300: 100: 100 Kg.ha^{-}

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

Soil texture	Soil separates (%)			pH (ds.m^{-1})	EC (mg.kg^{-1})	K availability (mg.kg^{-1})	P availability (mg.kg^{-1})	N availability matter (g.kg^{-1})	Organic equivalent.L^{-1})	Ca (ml
	Clay	Sand	Silt							
Sandy Loam	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.
										Bacillius sp
	$3.1\beta10^4$			$6.2\beta10^5$			*Types of fungi found in the soil of the plastic house			*Aspergillus florecenus*
										Penecillium sp.

^{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 leaves 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 Medicinal and Aromatic Plants Research Unit according to the method described by Cualutz *et al*. (1977). 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, 1980).

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	
	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.

Table 3 : Sources of active elements constituents for Joud SmartFert fertilizer,

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

Table 4 : Ingredients of dry bread yeast (*Sacchromyce cervisiae*) produced by Turkish company Lesaffre (Vanìk *et al.*, 200142).

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

Table 5 : 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 6 : Ingredients of seaweed extract (Alga Star).

Active components	Proportion	Active components	Proportion
N	1%	**Alginic acid**	10 %
K_2O	18%	**Organic matter**	45 %
Sulfur	3.1 %	**Natural growth regulators**	45 %
Peptin	3.1 %	**vitamins**	3.1 %
Mannitol	3.1%		

Studied traits

Traits of vegetative growth

Five plants were randomly selected from the middle line of the cultivation to measure the traits of vegetative growth and after completion of the spraying treatments with the 10-day bio-stimulators, then the average was calculated.

The average of Plant height (cm)

It was measured by using the measuring tape from the surface of the soil to the top of the plant after the end of the spraying treatments for the total vegetation of each plant of the measured experimental unit and calculate the average.

The average of fresh weight for leaves (g)

It was calculated for each plant of experimental unit and then the average was calculated.

The average of dry weight for leaves (g.plant^{-1})

The leaves of the plants were dried at the temperature in the ventilated room belonging to the Research Unit of the medical and aromatic plants after they were placed on pasteboard with the process of flipping the samples from one period to another until the total drought and the stability of the weight, then the dry weight of each plant of experimental unit was measured and calculate the average.

Average number of leaves per plant (leaf.plant^{-1})

It was calculated for each plant of experimental unit and then the average was calculated.

The yield of plastic house of the dried leaves on the basis of its area (kg.house^{-1})

It was calculated based on the area of the plastic house, which dimensions 9 × 54 m^2, which was the total number of plants in which was 600 plants according to the following equation:

The yield of plastic house from dried leaves (kg.house^{-1}) = the average of Plant yield from dry leaves × Total number of plants inside the plastic house (Mohammed, 2016).

Average of leaf area (cm^2)

Ten leaves were selected randomly for each plant, starting from the bottom of the plant to its upper end, the average area of the one leaf was calculated and then multiplied by the number of leaves per plant. The leaf area was calculated for five plants selected from each

experimental unit. The scanner was used by the Digimizer program loaded on a Dell computer and according to the method described by Smith and Read (1997), then calculated the result according to the following equation:

The leaf area of the plant (ds^2)

$$= \frac{The\ average\ area\ of\ the\ one\ leaf}{Number\ of\ leaves\ per\ one\ plant} \times 100$$

Estimation the content of total chlorophyll, a, and b in fresh leaves (mg. 100 g^{-1} fresh weight)

The content of total chlorophyll, a and b for fresh leaves was estimated at the Nutrition Lab belonging to Horticulture and Garden Landscape after the full leaves were selected from the plants of measured experimental unit. The concentration of chlorophyll a, b and total (after the completion of spraying treatments with bio-stimulators) was determined according Goodwin (Goodwin, 1976) method. The light absorption of the sample was then read by a spectrophotometer on 668 nm and 645 nm wavelengths, followed by the concentration of chlorophyll ($mg.L^{-1}$) according to the following equation (Goodwin, 1976):

Chlorophyll a ($mg.L^{-1}$) = 12.7D(663)-2.69D(645)

Chlorophyll b ($mg.L^{-1}$) = 22.9D(645)-4.68D(663)

Total chlorophyll ($mg.L^{-1}$) = {20.2D(645) + 8.02D (663)}.

D = reading the obtained chlorophyll photovoltaic density at wavelengths 663 and 645 nm. It was then converted into (mg. 100 g^{-1} fresh weight).

Results and Discussion

Results

The average of plant height (cm)

Table 7 indicates that there is a significant effect of the planting date in the average of plant height. The addition of Mycorrhiza fungal vaccine M1 showed a significant excelling on the treatment of without the addition of the Mycorrhiza fungal vaccine M0 to the root system of (87.17, 74. 19 cm), respectively. The bio-stimulators treatment C4 (seaweed extract) was excelled on all the bio-stimulators treatments where recorded the highest average of plant height of 89.31 cm, while the control treatment C0 (spraying with distilled water only) recorded the lowest plant height of 64.69 cm. The same table shows significant differences for bi-interaction between the planting date treatments and the Mycorrhiza fungal vaccine (D × M), where the interaction between the first planting date and the addition of the Mycorrhiza fungal vaccine (D1M1) recorded the highest average plant height (90.73 cm) compared to the interaction between the treatment of the second planting date and without the addition of the Mycorrhiza fungal vaccine (D2M0), which recorded a marked decrease in all the plant height of (69.62 cm). The interaction between the first planting date and the spraying with the bio-stimulators (D×C) was significantly affected, where the interaction treatment between the first planting date and the spraying with the bio-stimulators (seaweed extract) D1C4 gave the highest average for the plant height (94.17 cm) while the interaction treatment between the second planting date and the spraying with distilled water only (the control treatment) D2C0 recorded the lowest plant height (60.38 cm).The bi-interaction between the mycorrhiza fungal vaccine and bio-stimulators (M4C, M1C) showed a significant excelling, Where M1C4 treatment was characterized by recording the highest average of plant height (96.80 cm), while the bi-interaction treatment (M0C0) recorded a significant decrease in the plant height (60.26 cm) as shown in table 7. The results of the triple interaction between the study factors showed the significant excelling in the plant height, where the treatments of D1M1C4, D1M1C3 and D1M1C2 were excelled by giving them the highest plant height of (101.67, 100.17, 99.50 cm), respectively, compared to the treatment D2M0C0, which recorded a marked decrease in plant height of (52.43 cm) as shown in table 7.

Average of fresh weight for leaves of plant ($gm.plant^{-1}$)

Table 8 shows the existence of a significant difference between the treatments of the single study factors, individually in the rate of soft weight of plant leaves. The first date D1 differed from the second date D2 by giving it the highest average weight of (94 $g.plant^{-1}$) compared to the second date D2, which recorded (92.28 $g.plant^{-1}$).The treatment of the addition of the Mycorrhiza fungal vaccine M1 was excelled on the non-addition of the Mycorrhiza fungal vaccine M0 in the trait of average of fresh weight for leaves, which gave (97.29, 88.99 $g.plant^{-1}$), respectively. The treatments of the bio-stimulators showed significant differences between them. The treatment of C4 was excelled by giving it the highest average of fresh weight for leaves of plant (102.57 $g.plants^{-1}$) compared to C0 (control treatment), which recorded a significant decrease in the fresh weight of leaves by giving it (80.35 $g.plants^{-1}$) as shown in table 8. Bi-interaction treatments for the study factors was significantly affected the trait of the fresh weight of leaves. Both D1M1 and D2M1 treatments were characterized by recording the highest average of fresh

Table 7 : Effect of planting dates, Mycorrhiza and bio-stimulators in average of plant height (cm).

Planting dates	Mycorrhiza vaccine	Bio-stimulators					Interaction D x M
		C0	C1	C2	C3	C4	
D1	M0	68.00	75.17	80.30	86.67	86.67	78.76
	M1	70.00	82.30	99.50	100.17	101.67	90.73
D2	M0	52.43	71.67	72.43	74.60	76.97	69.62
	M1	68.33	79.17	90.00	88.67	91.93	83.62
							Effect of planting dates D
Interaction (Planting dates x Bio-stimulators) D x C	D1	69.00	78.73	89.9	91.92	94.17	84.74
	D2	60.38	75.47	81.22	81.63	84.45	76.62
							Effect of fungal Mycorrhiza vaccine M
Interaction (fungal Mycorrhiza vaccine x Bio-stimulators) M x C	M0	60.22	69.17	76.37	79.13	81.82	74.19
	M1	73.42	80.73	94.75	94.42	96.80	87.17
Effect of Bio-stimulators C		64.69	77.08	85.56	86.78	89.31	
L.S.D 0.05							
D×M×C	M×C	D×C	D × M	C	M		D
7.233	5.116	5.116	5.992	3.617	4.237		3.608

Table 8 : Effect of planting dates, Mycorrhiza and bio-stimulators in average of fresh weight for leaves of plant (g.plant^{-1}).

Planting dates	Mycorrhiza vaccine	Bio-stimulators					Interaction D x M
		C0	C1	C2	C3	C4	
D1	M0	74.83	86.33	93.67	94.00	97.20	89.21
	M1	87.33	95.50	98.83	102.67	109.67	98.80
D2	M0	73.57	84.00	92.33	94.67	99.30	88.77
	M1	85.67	90.33	100.33	98.50	104.10	95.79
							Effect of planting dates D
Interaction (Planting dates x Bio-stimulators) D x C	D1	81.08	90.92	96.25	98.83	103.43	94.00
	D2	79.62	87.17	96.33	96.58	101.70	92.28
							Effect of fungal Mycorrhiza vaccine M
Interaction (fungal Mycorrhiza vaccine x Bio-stimulators) M x C	M0	74.20	85.17	93.00	94.53	98.25	88.99
	M1	86.50	92.92	99.58	100.58	106.88	97.29
Effect of Bio-stimulators C		80.35	89.04	96.29	97.46	102.57	
L.S.D 0.05							
D×M×C	M×C	D×C	D × M	C	M		D
8.074	5.709	5.709	6.239	4.037	4.412		1.662

weight of (98.80, 95.79 g.plants^{-1}), while both D1M0 and D2M0 recorded the lowest average of fresh weight for leaves (89.21.88.77 g.plants^{-1}). Bi-interaction treatment D1C4 was characterized by recording it the highest average of fresh weight for leaves amounted of (103.43 g.plants^{-1}), while D1C0 and D2C0 treatments recorded the lowest average of fresh weight amounted of (81.08, 79.62 g.plants^{-1}). The M1C4 interaction treatment was

excelled on the other bi-interaction treatments by recording it the highest fresh weight for leaves (106.88 g.plant^{-1}) compared to the treatment M0C0, which recorded a decrease in the fresh weight of the leaves for the plant (74.20 g.plant^{-1}) as shown in table 8. Triple interaction treatments between the study factors (M × C × D) showed significant differences in the trait of fresh weight for leaves of plant. The treatment of D1M1C4 was excelled on the rest of the treatments by giving the highest fresh weight of leaves for plant (109.67 g.plant^{-1}), While the triple interaction treatments (D1M0C0 and D2M0C0) recorded the lowest averages fresh weight for leaves (74.83, 73.57 g.plant^{-1}), respectively as shown in table 8.

Dry weight for leaves of plant (g.plant^{-1})

Table 9 shows the significant effect of planting dates in the trait of dry weight for leaves of plant, where the first planting date D1 was excelled on the second planting date D2 in the average of dry weight for leaves of plant, which amounted of (46.12, 42.31 g.plant^{-1}), respectively. While the treatment of the addition of Mycorrhiza fungal vaccine to the root system of plant M1 was significantly excelled on the non-addition of the Mycorrhiza fungal vaccine M0, which amounted of (45.73, 42.31 g.plant^{-1}), respectively. The spraying treatment of with the bio-stimulators (seaweed extract) C4 showed a significant excelling on all treatments, which recorded the highest dry weight for leaves of plant amounted of (50.30 g.plant^{-1}) compared to the spraying treatment with distilled water only C0, which recorded a significant decrease in the dry weight of leaves was (34.98 g.plant^{-1}). As for the bi-interaction treatments, the interaction treatment between the first planting date and the addition of the Mycorrhiza fungal vaccine (D1M1) was significantly excelled by giving it the highest dry weight of (47.67 g.plant^{-1}), while the interaction treatment between the second planting date and the non-addition of the Mycorrhiza fungal vaccine for the root system D2M0 recorded the lowest average of dry weight for leaves was (40.85 g.plant^{-1}). The effect of bi-interaction between the planting dates and the bio-stimulators was significant in all treatments, where the bi-interaction treatment between the first planting date and spraying with the bio-stimulator (seaweed extract) D1C4 was excelled by recording the highest dry weight of (51.70 g.plant^{-1}) compared to the bi-interaction treatment between the second planting date and the spraying of the total vegetative with distilled water only D2C0, where a significant decrease in the average of dry weight for the leaves amounted of 33.30 g.plant^{-1}. The interaction treatments between the Mycorrhiza fungal vaccine and the spraying with bio-stimulators on the total vegetative were significantly affected in all treatments (M × C). The M1C4 treatment was significantly characterized in the trait of the average of dry weight for the leaves which amounted of (51.15 g.plant^{-1}) compared to the bi-interaction treatment M0C0, which recorded the lowest dry weight for leaves was (32.62 g.plant^{-1}). The triple interaction treatments between planting date, Mycorrhiza fungal, and the spraying with bio-stimulators (D × M × C) had a significant effect on all treatments. Where the triple interaction treatments between the planting date, the addition of the Mycorrhiza fungal vaccine to the total vegetative and the spraying with seaweed extract D1M1C4 was significantly excelled on the rest of the treatments where recorded the highest dry weight amounted of (53.20 g.plant^{-1}), while the D2M0C0 treatment recorded the lowest dry weight of 29.83 g.plant^{-1}.

Average number of leaves per plant (leaf.plant^{-1})

Table 10 indicates that there are significant differences between the treatments of the single study factors in the trait of the average total number of leaves in the plant. The first planting date D1 was significantly excelled by recording the highest average of the total number of leaves for the plant which amounted of (160.37 leaf.plant^{-1}). While observed a decrease in the total number of leaves for the second planting date D2 (138.87 leaf.plant^{-1}). The M1 treatment showed significant excelling by giving it the highest average of total number of leaves of (169.21 leaf.plant^{-1}) compared to the M0 treatment, which recorded the lowest average of the total number of leaves in the plant reached (130.04 leaf.plant^{-1}). The C4 treatment was characterized by giving it the highest average of total number of leaves in the plant (185.26 leaf.plant^{-1}) compared with the control treatment C0 which showed a significant decrease in the total number of leaves for the plant reached (111.26 leaf.plant^{-1}) as shown in table 10. Bi-interaction treatments for the study factors showed a significant differences in the trait of number of leaves for the plant. Both the D1M1, D1C4 and M1C4 bi-interaction treatments excelled by recording it the highest number of leaves for plant of (183.40, 191.67, 198.84 leaf.plant^{-1}), respectively. while D2M0, D2C0 and M0C0 treatments showed a significant decrease in the total number of leaves for plant (127.33, 95.17, 82.84 leaf.plant^{-1}), respectively as shown in table 10. The D1M1C4 treatment was characterized by giving it the highest average of the total number of leaves of (216.67 leaf.plant^{-1}) compared to the D1M0C0 and D2M0C0 triple interaction treatments, which showed the lowest average of total number of leaves of (90.67, 75.00 leaf.plant^{-1}), respectively as shown in table 10.

Table 9: Effect of planting dates, Mycorrhiza and bio-stimulators in average of dry weight for leaves of plant (g.plant^{-1}).

Planting dates	Mycorrhiza vaccine	Bio-stimulators					Interaction D x M
		C0	C1	C2	C3	C4	
D1	M0	35.40	42.67	46.60	47.93	50.20	44.56
	M1	37.93	47.23	50.67	49.33	53.20	47.67
D2	M0	29.83	36.17	44.03	45.50	48.70	40.85
	M1	36.77	39.17	48.17	45.70	49.10	43.78
							Effect of planting dates D
Interaction (Planting dates x Bio-stimulators) D x C	D1	36.67	44.95	48.63	48.63	51.7	46.12
	D2	33.30	37.67	46.10	45.60	48.90	42.31
							Effect of fungal Mycorrhiza vaccine M
Interaction (fungal Mycorrhiza vaccine x Bio-stimulators) M x C	M0	32.62	39.42	45.32	46.72	49.45	42.70
	M1	37.35	43.20	49.42	47.52	51.15	45.73
Effect of Bio-stimulators C		34.98	41.31	47.37	47.12	50.30	
L.S.D 0.05							
D×M×C	M×C	D×C	D × M	C	M		D
2.894	2.049	2.049	1.901	1.447	1.344		1.849

Table 10 : Effect of planting dates, Mycorrhiza and bio-stimulators in average of number of leaves per plant (leaf.plant^{-1}).

Planting dates	Mycorrhiza vaccine	Bio-stimulators					Interaction D x M
		C0	C1	C2	C3	C4	
D1	M0	90.67	130.33	144.00	155.00	166.67	137.33
	M1	150.00	175.00	191.67	183.67	216.67	183.40
D2	M0	75.00	119.00	138.33	146.67	157.67	127.33
	M1	120.33	143.67	179.00	168.00	185.00	155.00
							Effect of planting dates D
Interaction (Planting dates x Bio-stimulators) D x C	D1	120.34	152.67	167.84	169.34	191.67	160.37
	D2	95.17	131.34	150.17	150.84	166.84	138.87
							Effect of fungal Mycorrhiza vaccine M
Interaction (fungal Mycorrhiza vaccine x Bio-stimulators) M x C	M0	82.84	122.67	137.17	147.84	159.67	130.04
	M1	132.67	161.34	180.84	172.34	198.84	169.21
Effect of Bio-stimulators C	111.26	145.76	166.51	166.59	185.26		
L.S.D 0.05							
D×M×C	M×C	D×C	D × M	C	M		D
25.070	17.610	17.610	24.900	12.450	17.730		20.770

The average yield of the plastic house of dry leaves on the basis of its area (kg.house^{-1})

Table 11 shows that there is a significant effect of planting dates in the trait of the yield of plastic house for dry leaves based on the area of the house. The first planting date of D1 was excelled by giving it the highest yield from dry leaves amounted of (27.68 kg.house^{-1}), While the second planting date D2 recorded a decrease

in the yield of dry leaves of (26.02 kg.house^{-1}). The addition of the Mycorrhiza fungal vaccine had a significant effect in the same trait, where the treatment of adding the Mycorrhiza fungal vaccine M1 to the root system was significantly excelled by giving it the highest yield of dry leaves of (27.87 kg.house^{-1}), while the treatment of the non-addition of the Mycorrhiza fungal vaccine to the root system M0 recorded a decrease in the same trait above amounted of (26.40 kg.house^{-1}). The treatments of spraying total vegetative with bio-stimulators. The C4 treatment was significantly characterized by recording it the highest average of total yield from dry leaves in plastic house of (30.66 kg.house^{-1}), while C0 treatment recorded a decrease in the average of total yield from dry leaves in the plastic house reached (21.48 kg.house^{-1}) as shown in table 11. The bi-interaction treatments of D1M1, D1C4 and M1C4 showed a significant increase by giving it the highest yield of plastic house from dried leaves of (28.62, 31.02, 31.14 kg.house^{-1}), respectively. The bi-interaction treatments of D2M0, D2C0 and M0C0 showed a significant decrease in the average yield of dry leaves for plastic house (24.86, 20.05, 19.96 kg.house^{-1}), respectively as shown in table 11. The results of the same table showed a significant excelling for the triple interaction treatment D1M1C4 by giving it the highest average of total yield of the dry leaves for the plastic house amounted to (31.92 kg.house^{-1}) compared with the triple interaction treatment D2M0C0, which recorded the lowest average of the plastic house for dry leaves (18.85 kg.house^{-1}) as shown in table 11.

Average of total leaf area (dm^2)

Table 12 shows that there is a significant effect of planting dates in the percentage of total leaf area. The first planting date D1 was significantly excelled on the second planting date by giving it the highest average of the total leaf area of 21.72 dm^2, while the second planting date D2 recorded the lowest average of the total leaf area of 18.80 dm^2. The Mycorrhiza vaccine was significantly affected in the same trait where the treatment of the addition of Mycorrhiza vaccine M1 was significantly excelled on the non-addition of the Mycorrhiza fungal vaccine to root system of plant M0 (22.33, 18.19 dm^2), respectively. The spraying treatment with bio-stimulators (seaweed extract) C4 was significantly excelled on the other bio-stimulators treatments by giving it the highest average of total leaf area of (26.57 dm^2) compared to the spraying treatment with distilled water only (control treatment) C0, which recorded a significant decrease in the average of total leaf area (11.58 dm^2). The bi-interaction treatment between the planting date and Mycorrhiza (D×M) was significantly affected, the bi-interaction treatment D1M1 was characterized by recording it the highest average of total leaf area amounted (24.11 dm^2) compared to D2M0 and D1M0 treatments, which recorded the lowest averages of total leaf area of (17.05, 19.31 dm^2), respectively. The interaction treatment in the same table between planting date and spraying with bio-stimulators (D × C) showed a significant effect evident in the same trait above Where the D1C4 treatment was significantly excelled in the total leaf area amounted of (28.86 dm^2), while D2C0 bi-interaction treatment recorded the lowest average of the total leaf area of 9.84 dm^2. Both M1C2, M1C4 and M0C4 bi-interaction treatments were excelled by recording it the highest total leaf area of (27.99, 27.05, 26.08 dm^2) compared to the bi-interaction treatments of M0C0, which recorded a decrease in total leaf area of (9.63 dm^2) as shown in table 12. The triple interaction treatments between the planting dates, the Mycorrhiza fungal vaccine and the bio-stimulators treatment showed a significant effect represented by the superiority of both the D1M1C2 and D1M1C4 triple interaction treatment, which had a significant effect on the recording of the highest total leaf area of (29.93, 29.77 dm^2). While the D3M1C0, D1M0C0 and D2M0C0 triple interaction treatments recorded a decrease in total leaf area (11.18, 10.75, 8.50 dm^2) as shown in table 12.

The concentration of chlorophyll a in the leaves (mg. 100 g^{-1} fresh weight)

Table 13 shows significant differences between the treatments of each of the study factors individually in the trait of concentration of chlorophyll a in leaves. Treatment D1 recorded the highest concentration of chlorophyll a in leaves of (4.01 mg.100 g^{-1}fresh weight) compared to D2 treatment, which recorded the lowest concentration of chlorophyll a in leaves of (3.08 mg.100 g^{-1} fresh weight). The M1 treatment was significantly excelled by recording it the highest concentration of chlorophyll a in leaves of (3.96 mg.100 g^{-1} fresh weight) compared to the treatment M0, in which the concentration of chlorophyll a was low amounted of (3.83 mg.100 g^{-1} fresh weight). The C3 and C4 treatments were excelled by recording it the highest chlorophyll concentration of (4.37, 4.30 mg.100 g^{-1} fresh weight), respectively, while C0 treatment recorded a decrease in the concentration of chlorophyll a in leaves of (2.96 mg.100 g^{-1} fresh weight) as shown in table 13. Bi-interaction treatment were significantly affected in the same trait above. The D1M1, D1C4, M1C2 and M1C4 treatment showed the highest concentration of chlorophyll a in leaves (4.07, 4.53, 4.48, 4.38 mg.100 g^{-1} fresh weight), while D2M0, D1C0, D2C0 and M1C0 treatment showed a decrease in chlorophyll concentration in leaves of (3.72,

Table 11 : Effect of planting dates, Mycorrhiza and bio-stimulators in average yield of the plastic house of dry leaves on the basis of its area (kg.house^{-1}).

Planting dates	Mycorrhiza vaccine	Bio-stimulators					Interaction D x M
		C0	C1	C2	C3	C4	
D1	M0	21.24	25.60	27.96	28.76	30.12	26.74
	M1	22.76	28.34	30.44	29.66	31.92	28.62
D2	M0	18.85	23.50	26.42	27.30	28.22	24.86
	M1	21.06	26.62	29.23	28.60	30.36	27.28
							Effect of planting dates D
Interaction (Planting dates x Bio-stimulators) D x C	D1	22.00	26.97	29.20	29.21	31.02	27.68
	D2	19.96	25.06	27.83	27.95	29.29	26.02
							Effect of fungal Mycorrhiza vaccine M
Interaction (fungal Mycorrhiza vaccine x Bio-stimulators) M x C	M0	20.05	24.55	27.69	28.53	29.67	26.40
	M1	21.74	27.48	29.84	29.13	31.14	27.87
Effect of Bio-stimulators C		21.48	26.76	29.01	28.91	30.66	
L.S.D 0.05							
D×M×C	M×C	D×C	D × M	C	M		D
1.814	0.924	0.924	0.650	0.654	0.460		1.307

Table 12 : Effect of planting dates, Mycorrhiza and bio-stimulators in average of Total leaf area (dm^2).

Planting dates	Mycorrhiza vaccine	Bio-stimulators					Interaction D x M
		C0	C1	C2	C3	C4	
D1	M0	10.75	16.04	19.21	22.77	27.79	19.31
	M1	15.90	22.93	29.77	22.04	29.93	24.11
D2	M0	8.50	14.01	18.40	14.98	24.37	17.05
	M1	11.18	19.40	26.20	21.78	24.16	20.54
							Effect of planting dates D
Interaction (Planting dates x Bio-stimulators) D x C	D1	13.33	19.49	24.49	22.41	28.86	21.72
	D2	9.84	16.71	22.30	20.88	24.27	18.80
							Effect of fungal Mycorrhiza vaccine M
Interaction (fungal Mycorrhiza vaccine x Bio-stimulators) M x C	M0	9.63	15.03	18.81	21.38	26.08	18.19
	M1	13.54	21.17	27.99	21.91	27.05	22.33
Effect of Bio-stimulators C		11.59	18.10	23.40	21.65	26.57	
L.S.D 0.05							
D×M×C	M×C	D×C	D × M	C	M		D
3.881	2.634	2.634	3.726	1.863	2.745		0.645

3.01, 2.90, 2.63 mg.100 g^{-1} dry weight), respectively as show in table 13. The triple interaction treatment between the study factors had a significant effect on the concentration of chlorophyll a in the leaves, where the D1M1C2 treatment recorded the highest concentration of chlorophyll a in leaves of (4.58 mg.100 g^{-1} fresh weight), while the triple interaction treatments D1M1C0 and D2M1C0 recorded the lowest concentration of

Table 13 : Effect of planting dates, Mycorrhiza and bio-stimulators in the concentration of chlorophyll a in the leaves (mg. 100 g^{-1} fresh weight).

Planting dates	Mycorrhiza vaccine	Bio-stimulators					Interaction D x M
		C0	C1	C2	C3	C4	
D1	M0	3.33	3.61	3.89	4.38	4.49	3.94
	M1	2.69	4.05	4.58	4.47	4.56	4.06
D2	M0	3.23	3.46	3.64	4.08	4.21	3.72
	M1	2.57	3.87	4.38	4.26	4.30	3.88
							Effect of planting dates D
Interaction (Planting dates x Bio-stimulators) D x C	D1	3.01	3.83	4.24	4.43	4.35	4.01
	D2	2.90	3.67	4.01	4.17	4.26	3.80
							Effect of fungal Mycorrhiza vaccine M
Interaction (fungal Mycorrhiza vaccine x Bio-stimulators) M x C	M0	3.28	3.54	3.77	4.23	4.35	3.83
	M1	2.63	3.96	4.48	4.37	4.38	3.96
Effect of Bio-stimulators C		2.96	3.75	4.13	4.30	4.37	
L.S.D 0.05							
D×M×C	M×C	D×C	D × M	C	M		D
0.198	0.142	0.142	0.160	0.099	0.114		0.161

Table 14 : Effect of planting dates, Mycorrhiza and bio-stimulators in the concentration of chlorophyll b in the leaves (mg. 100 g^{-1} fresh weight).

Planting dates	Mycorrhiza vaccine	Bio-stimulators					Interaction D x M
		C0	C1	C2	C3	C4	
D1	M0	1.65	1.81	1.94	2.19	2.25	1.97
	M1	1.34	2.03	2.29	2.23	2.28	2.03
D2	M0	1.62	1.73	1.82	2.04	2.11	1.86
	M1	1.28	1.93	2.19	2.16	2.11	1.95
							Effect of planting dates D
Interaction (Planting dates x Bio-stimulators) D x C	D1	1.50	1.92	2.12	2.21	2.27	2.00
	D2	1.45	1.83	2.01	2.10	2.15	1.91
							Effect of fungal Mycorrhiza vaccine M
Interaction (fungal Mycorrhiza vaccine x Bio-stimulators) M x C	M0	1.64	1.77	1.88	2.12	2.18	1.92
	M1	1.31	1.98	2.24	1.75	2.01	1.99
Effect of Bio-stimulators C		1.48	1.87	2.06	2.16	2.21	
L.S.D 0.05							
D×M×C	M×C	D×C	D × M	C	M		D
0.098	0.070	0.070	0.080	0.049	0.056		0.081

Table 15 : Effect of planting dates, Mycorrhiza and bio-stimulators in the total concentration of chlorophyll in leaves (mg. 100 g^{-1} fresh weight).

Planting dates	Mycorrhiza vaccine	Bio-stimulators					Interaction D x M
		C0	C1	C2	C3	C4	
Dl	M0	5.00	5.42	5.83	6.57	6.74	5.91
	M1	4.03	6.09	6.88	6.68	6.84	6.10
Dl	M0	4.86	5.20	5.46	6.13	6.32	5.59
	M1	3.85	5.80	6.58	6.40	6.48	5.82
							Effect of planting dates D
Interaction (Planting dates x Bio-stimulators) D x C	Dl	4.52	5.76	6.36	6.63	6.79	6.01
	D2	4.36	5.50	6.02	6.27	6.40	5.71
							Effect of fungal Mycorrhiza vaccine M
Interaction (fungal Mycorrhiza vaccine x Bio-stimulators) M x C	M0	4.93	5.31	5.65	6.35	6.53	5.75
	M1	3.94	5.95	6.73	6.54	6.66	5.96
Effect of Bio-stimulators C		4.44	5.63	6.19	6.45	6.60	
L.S.D 0.05							
D×M×C	M×C	D×C	D × M	C	M		D
0.298	0.211	0.211	0.243	0.149	0.170		0.240

chlorophyll a in the leaves was (2.69, 2.57 mg.100 g^{-1} fresh weight), respectively as shown in table 13.

The concentration of chlorophyll b in the leaves (mg. 100 g^{-1} fresh weight)

Table 14 shows significant differences between the treatments of the study factors individually in the trait of the concentration of chlorophyll b in the leaves. Both D1, M1 and C4 treatments characterized by giving it the highest concentrations of chlorophyll b in soft leaves (2.00,1.99, 2.21 mg, 100 g^{-1} fresh weight), respectively. In contrast, both D2, M0 and C0 recorded the lowest concentration of chlorophyll b in the leaves (1.91, 1.99, 1.48 mg.100 g^{-1} fresh weight), respectively as shown in table 14. Bi-interaction treatment between the study factors were affected in the same trait studied above. The D1M1, D1C4, M1C2, M1C4 and M1C3 treatments were characterized by recording it the highest concentration of chlorophyll b in the leaves (2.03, 2.27, 2.14, 2.23, 2.19 mg.100 g^{-1}), respectively, while bi-interaction treatments D2M0, D1C0, D2C0 and M1C0 recorded the lowest concentration of chlorophyll b in leaves was (1.86, 1.50, 1.45, 1.31 mg.100 g^{-1} fresh weight), respectively as shown in table 14. The triple interaction treatments were differed between the study factors (D × M × C) in the concentration of chlorophyll b in the fresh leaves. The triple interaction treatments D1M1C2 was excelled by giving it the highest concentration of chlorophyll b in the fresh leaves content which amounted of (29.2 mg.100 g-1 fresh weight), respectively, while the D1M1C0 and D2M1C0 triple interaction recorded the lowest concentration of chlorophyll b in the fresh leaves of (1.34, 1.28 fresh weight), respectively, as shown in table 14.

Total concentration of chlorophyll in leaves (mg. 100 g^{-1} fresh weight)

Table 15 shows significant differences between the study factors individually in total chlorophyll concentration of leaves. Both D1, M1 and C4 treatments was excelled by recording it the highest concentration of total chlorophyll for leaves (6.01, 5.96, 6.60 mg.100 g^{-1} fresh weight) respectively. while D2, M0, and C0 treatments recorded the lowest concentration of total chlorophyll in fresh leaves (5.71, 5.75, 4.44 mg. 100 g^{-1} fresh weight) as shown in table 15. The bi-interaction treatment between planting dates and Mycorrhiza (DXM) showed significant differences in the same studied traits above. The bi-interaction treatment D1M1 characterized by recording it the highest total chlorophyll concentration of leaves (6.10 mg.100 g^{-1} fresh weight). While the bi-interaction treatment D2M1 and D2M0 recorded a decrease in total chlorophyll concentration of leaves (5.82, 5.59 mg.100 g^{-1} soft weight).The bi-interaction treatments

D1C4, D1C3, M1C2 and M1C4 were significantly excelled in their concentration of total chlorophyll in leaves (6.79, 6.63, 6.73, 6.66 mg.100 g^{-1} soft weight), while the D1C0, D2C0 and M1C0 bi-interaction treatments recorded a decrease in total chlorophyll concentration of (4.52, 4.36, 3.94 mg. 100 g^{-1} fresh weight) as shown in table 15. The triple interaction between the first planting date and the addition of Mycorrhiza vaccine to the root system and spraying with bio-stimulators (Chitosan) D1M1C2 was significant affected in the same trait above, which amounted of (6.88 mg. 100 g^{-1} fresh weight).While the triple interaction treatment D1M1C0 and D2M1C0 recorded a significant decrease in total chlorophyll concentration in leaves of (4.03, 3.85 mg. 100 g^{-1} fresh weight) as shown in table 15.

Discussion

Planting date is considered a one of the factors determining the growth of *Stevia rebaudiana* Bertoni. plant and its development in the presence of the optimal environmental conditions during the period of plant growth, which are positively reflected in obtaining the highest rate of growth of the plant and its yield of dried leaves (Maheshwar, 2005). Khan *et al.* (2012) were found that the planting date of plant in the permanent field was a prominent step in determining the quantitative and qualitative traits of medicinal plants. The resulting increase in the rates of all vegetative traits at the first planting date (D1) as shown in Tables 7-15 may be due to the positive effect of the maximum and minimum temperatures, relative humidity and the number of hours of solar brightness and the relationship between them that were optimal for plant growth until harvest during the first planting date D1, as shown in table 2. On the other hand, the second planting date D2, which was characterized by rising maximum and minimum temperatures, with a decrease in the relative humidity and an increase in the number of hours of solar brightness during the period of the start of plant growth until harvest as shown in table 2, which had an effect on the decrease in the studied traits of vegetative growth at the second planting date as shown in tables 7-15. Beement (2012) found that the best planting date for cultivating of *S. rebaudiana* Bertoni plant in Ethiopia, which their temperatures ranging from 6-25°C for the beginning of seedling growth in the field, which was positively reflected in the increase in vegetative growth indicators (plant height, fresh and dry weight of leaves, number of leaves, stem diameter, concentration of total chlorophyll) and thus increase the dry yield of leaves (Suhail, 2013). The results obtained in this study agree with those found by Khan *et al*. (2012), Taleie *et al*. (2012) and Maheshwar (2005). As for excelling the treatment of M1 to most traits of the vegetative growth in this study as shown in Table (7-15) compared to the control treatment M0, Which recorded a significant decline in all averages of vegetative growth due to the strong reinforcement of the root system of *S. rebaudiana* Bertoni. plant, as a result of its strongly colonization of the root zone, Which resulted in a striking balance between the root system under soil and the vegetative parts over the soil. The Mycorrhiza fungal have increased the absorption of water and nutrients for growth, which has been positively reflected in improved growth and increased production of biomass in *S. rebaudiana* plants (Harrier and Sawezak, 2000; Singh *et al*., 2000; Rai *et al*., 2001; Varma *et al*., 2001; Kumari *et al*., 2003; Waller *et al*., 2005). In addition, An increase in the rate of photosynthesis process in plants whose roots are treated by it (Wu and Xia, 2006). The results of this study agree with Hoseini et al. (2015), Earanna (2007) and Hemavathi *et al* (2006). Plant bio-stimulators are divided into two forms: either naturally created such as amino acids, yeast extract, seaweed extract, Chitosan, hormones and plant growth regulators or artificially synthesized such as synthetic hormones, phenolic compounds, inorganic salts and essential elements (Rafiee *et al*., 2016). The results showed that there was a significant effect on the factors of vegetative growth in all the vegetative growth characteristics of this study as shown in tables 7-15. Where the treatment of C4 was significantly improved by giving it a significant increase in vegetative growth traits in tables 7-15. While C0 treatment recorded a decrease in all traits of vegetative growth as shown in tables 7-15. The increase in C4 treatment was attributed all traits of vegetative growth compared to C0 treatment (control), which recorded a decrease in most vegetative traits in this study to the chemical composition of the seaweed extract as shown in table 6. The seaweed extract had a nutritious effect for its containment of micro and macronutrients, acting as fertilizer to the side of its other roles of hormonal effect, which is one of the main reasons for effective biochemical stimulation in plant groups. In addition to that the seaweed extract containing on hormone-like substances such as sterols and polyamins, as well as containing Cytokinins, Gibberellins, Auxins, Abscisic acid (Craigie, 2011) and it has an effective anti-stress effect as it contains anti-stress and anti-oxidative substances and internal gene organizations that Self-response to stress (Calvo *et al*., 2014). The seaweed extract contains micro-elements such as Cu, Mo, B, Co, as well as the macro-elements such as Auxins, Gibberellins and Cytokinins. The foliar spraying

of the seaweed extract increases both the absorption of the elements and the susceptibility of root growth, stem thickness and most vegetative growth indicators (Jensen, 2004). The results of this experiment agree with Saif *et al*. (2014), Fayza *et al*. (2015), Salama *et al*. (2016) found that the use of seaweed extracts as foliar application, which significantly increased the vegetative growth indicators of the plant. In this study, we can explain the superiority of the D1M1 bi-interaction treatment in the events of increase in all traits of vegetative growth as shown in tables 7-15. The results obtained in this study on the superiority of the bi-interaction treatment of D1C4 in all traits of vegetative growth (tables 7-15) were due to the effect of the interaction between the first planting date D1 and the environmental conditions that prevailed in it, which were closer to the optimum from the beginning of the seedlings cultivation until the harvest date as shown in table 2, which combined their effect with the components of seaweed extract C4 as shown in table 6 when the plants treated with spray on the total vegetative, which explains the superiority of the bi-interaction treatment D1C4 in all traits of vegetative growth. The superiority of the bi-interaction treatment (M1C4) in most vegetative growth indicators as shown in tables 7-15 was due to the use of seaweed extract which stimulated the growth of the hypha of Mycorrhiza fungus, Which positively reflected on the absorption process of nutrients from the soil and converted through these fungal hyphae from the root to the Ariel parts of stevia plants, which gave an increase in the growth and yield of the plant through the effect of fungal vaccine to increase the root area of the plant and thus facilitate the penetration of soil as well as increased activity of enzymatic secretions of the existing Mycorrhiza in the root or through the hyphae. Mycorrhiza characterized by improving the growth of the plant through its secretion by the fungus of growth-regulating substances such as Auxins and Gibberellins, as well as the containing of seaweed extract of hormones such as Auxins, Gibberellins and Cytokinins (Jensen, 2004; Smith and Read, 1997; Barker and Tagu, 2000; Kuwada *et al*., 2006). The results of M1C4 treatment in this study agree with Suhail (2013). The superiority of the M1C2 treatment in both traits of the total leaf area and the concentration of a , b and total chlorophyll in fresh leaves as shown in tables 12-15 was due to the action of Chitosan in photosynthesis, which was reflected in the increase in the total leaf area and the concentration of total, a and b chlorophyll in fresh leaves, as well as that the Mycorrhiza fungal vaccine worked to increase the production of growth stimulators, which caused the increase in growth as well as the work on the recycling of nutrient elements and supplying plant by its growth requirements and increased the amount of water supply for the plant, Thus giving the plant resistance to environmental stress (Lowe *et al*., 2012; Vestberg and Cassells, 2009). The result of M1C2 treatment agrees with Lowe *et al*. (2012). The superiority of the triple interaction treatment (D1M1C4) is attributed in most traits of vegetative growth as shown in tables 7-12 to the fact that the use of seaweed extract has stimulated the growth of mycorrhiza hypha, which has been positively reflected in the absorption and conversion of nutrients from the soil through those fungal hypha from the root system to the Ariel part, which led to an increase in the growth of plant and their yield of leaves by the effect of fungal vaccine to increase the root area of the plant and thus facilitate the penetration of soil, as well as the seaweed extract worked to increase the activity of enzymatic secretions of the fungus Mycorrhiza, which are produced within the root of the host plant by the hypha fungal. The Mycorrhriza fungus improves the growth of the plant in the term of what is secreted by the fungal hypha of growth regulating substances such as Auxins, Gibberellins and cytokinins as well as the seaweed extract content of hormones such as Auxins, Gibberellins and cytokinins (Jensen, 2004; Smith and Read, 1997; Barker and Tagu, 2000; Kuwada *et al*., 2006). Triple interaction treatment D1M1C2 showed a significant excelling in both plant height, total leaf area and concentration of a,b and total chlorophyll in soft leaves as shown in tables 7 and 12-15. The cause is probably due to Chitosan which is a natural bio-stimulator that activates and increases nutrient absorption, chlorophyll content, photosynthesis and cell expansion (Malekpoor *et al*., 2016; Salachna *et al*., 2017). The catalytic effect of Chitosan in plant growth can be attributed to increased availability and absorption of water and essential nutrients and has also worked to increase the activity of enzymes in plants (Guan *et al*., 2009). The addition of the mycorrhiza vaccine in the triple interaction treatment has worked to increase the absorption of water and nutrients from the root system to the total vegetative, which has had a significant overlap in the increase in photosynthesis and increase the total leaf area in the plant. this confirms that the increase in the concentration of both chlorophyll a and b and total in fresh leaves as shown in tables 12-15 as well as the environmental conditions that prevailed in the first planting date D1 as shown in table 2, it also had an active role in vegetative growth of the plant and increased development of the severity of infection with mycorrhiza of the root system, which had a clear effect on the increase in concentration of total, a and b chlorophyll as shown in tables 12-15.

Conclusion

In this experiment, we found that the best treatment D1M1C4 because it impact vegetative growth characters while the treatment D1M1C2 impact the concentration of chlorophyll a,b and total in *Stevia rebaudiana* Bertoni. plant in Iraq.

References

Ahmed, B., M. Hossain, R. Islam, A. K. Saha and A. Mandal (2011). A review on natural sweetener plant stevia having medicinal and commercial importance. *Agro. Glasnik.*, **73**: 75-91.

Abdel-Fattah, D. A., W. E. Eweda, M. S. Zayed and M. K. Hassanein (2013). Effect of carrier materials, sterilization method, and storage temperature on survival and biological activities of *Azotobacter chroococcum* inoculants. *Annals of Agricultural Science*, **58(2)** : 111–118.

Al-Dulaimi, A. F. Z. (2012). Effect of foliar application with yeast extract and Amino–Quelant K in growth and yield of *Vitis vinifera* Black Hamburg.*PhD thesis*. Faculty of Agriculture, University of Baghdad. Pp. 144.

Al-Rawi Kh. M. A. A. Khalfalah (1980). Design and analysis of agriculture experiments collage of Agriculture & Frosty. Mosul University. Higher Education Printing House, pp. 488.

Aladakatti, Y. R., Y. B. Palled, M. B. Chetti, S. I. Hallkatti, S. C. Alagundagi, P. L. Patil, V. C. Patil and A. D. Janawade (2012). Effect of nitrogen, phosphorus and potassium levels on growth and yield of stevia (*Stevia rebaudiana* Bertoni.). *Karnataka J. Agric. Sci*., **25 (1)** : 25-29.

Barker, S. J. and D. Tagu (2000). The roles of auxins and cytokinins in mycorrhizal symbiosis. *J. Plant growth Regul*., **19(2)**: 144-154.

Beemnet, M. K., Z. D. Zigene, W. Kebede and H. Gebremeske (2012). Performance of Stevia (*Stevia rebaudiana* Bertoni) For Morphological and economic traits under different ecologies of Ethiopia. *J. AD*. **2 (2)**:1-16.

Calvo, P., L. Nelson and Klo J.W. Epper (2014). Agricultural uses of plant biostimulants. *Plant Soil*, **383(1-2)**: 3-41.

Chalutz, E., M. Lieberman and H. D. Sisler (1977). Methionine Induced ethylene production by *Penicillium digitatum*. *Plant Physiol*., **60**: 402-406.

Craigie, J. S. (2011). Seaweed extract stimuli in plant science and agriculture. *J. Appl. Phycol*., **23** : 371–393.

Earanna, N. (2007). Response of *Stevia rebaudiana* to biofertilizers. *Karnataka J. Agric. Sci*., **20(3)**:616-617.

Farooqi, A. A. and B. S. Sreeramu (2004). *Cultivation of Medicinal andAromatic Crops*. Universities Press (India) Limited, Hyderabad ,India pp. 344.

Fayza, H., N. Mohamed, U. El-Segai and E. A. Seleem (2015). Response of *Calendula officinalis* L. Plants to Growth Stimulants under Salinity Stress. *American-Eurasian J. Agric. and Environ. Sci*., **15(9)**: 1767-1778.

Goodwin, T. W. (1976). *Chemistry and biochemistry of plant pigments*. Volumes **1** and **2**. Chemis. and bioche.of plant pigments. Volumes 1 and 2. (Ed. 2).PP1-37.

Goyal, S. and R. Samsher (2010). Stevia abio-sweetener: A review. *Inter. J. Food Scienc and Nutr.*, **61**: 1–10.

Górnik, K., M. Grzesik and B. R. Duda (2008). The effect of chitosan on rooting of grapevine cuttings and on subsequent plant growth under drought and temperature stress. *Journal of Fruit and Ornamental Plant Research*, **16**: 333-343.

Guan Ya-jing, Jin Hu, Xian-ju Wang and Chen-xia Shao (2009). Seed priming with chitosan improves maize germination and seedling growth in relation to physiological changes under low temperature stress. *Journal of Zhejiang University Science*, **10(6)** : 427-433.

Harrier, L. A. and J. Sawczak (2000). Detection of the 3-phosphoglycerate kinase protein of *Glomus mosseae*. *Mycorrhiza*, **10**: 81-86.

Hemavathi, V. N., B. S. Sivakumr, C. K. Suresh and N. Earanna (2006). Effect of *Glomus fasciculatum* and plant growth promoting rhizobacteria on growth and yield of *Ocimum basilicum*. *Karnataka Journal of Agricultural Sciences*, **19** : 17–20.

Hoseini, R. Z., E. M. Goltapeh and S. Kalatejari (2015). Effect of bio-fertilizer on growth, development and nutrient content (leaf and soil) of *Stevia rebaudiana* Bertoni. *J. Crop Prot*., **4** : 691-704.

Jensen, E. (2004). Seaweed; fact or fancy. Published by Moses the Midwest Organic and Sustainable Education. *From the broad Caster*, **12(3)**: 164-170.

Lowe, A., S. M. R. McArdle and A. C. Cassells (2012). Effect of AMF-and PGPR root inoculation and a foliar chitosan spray in single combined treatments on powdery mildew disease in strawberry. *Agriculturral and Food Scince*, **21** :28-38.

Kaschuk, G., T. W. Kuyper, P. A. Leffelaar, M. Hungria and K. E. Giller (2009). Are the rates of photosynthesis stimulated by the carbon sink strength of rhizobial and arbuscular mycorrhizal symbioses?. *Soil Biol. Biochem*., **41** : 1233–1244.

Khan, A. R., S. H. Chowdhury and M. M. Karim (2012). Effect of date of planting on the growth and leaf yield of Stevia (*Stevia rebaudiana*). *J. Bangladesh Agril. Univ.*, **10(2)** : 205–210.

Kim, M., Y. S. Yeon, S. H. Myung, H. Choi, Y. Park, S. Ho, K. Kim, C. Choi and W. Jung (2005). Production of chitin and chitosan from the exoskeleton of adult two spotted field crickets (*Gryllus bimaculatus*). *Entomological Research*, **47(5)** : 279-285.

Kumari, R., H. Kishan, Y. K. Bhoon and A. Varma (2003). Colonization of cruciferous plants by *Piriformospora indica*. *Current Science*, **85** : 1672-1674.

Kuwada, K. L., S. Wamocho, M. Utemura, L. Matsushita and T. Ishi (2006). Effect of red green algae extra on hyphal growth of arbuscular mycorrhizal fungi and on mycorrhizal development and growth of papaya and passion fruit. *Agron. J.*, **98(5)** :1340–1344.

Maheshwar, H. M. (2005). Effect of different levels of nitrogen and dates of planting on growth and yield of Stevia (*Stevia rebaudiana* Bert.). *MScThesis*. Department of horticulture college of agriculture, Dharwad university of agricultural sciences, Dharwad. PP.66 .

Malekpoor , F., A. G. Pirbalouti and A. Salimi (2016). Effect of foliar application of chitosan on morphological and physiological characteristics of basil under reduced irrigation. *Res. on Crops*, **17 (2)** : 354-359.

Mandal, S., H. Evelin, B. Giri, V. P. Singh and R. Kapoor (2013). Arbuscular mycorrhiza enhances the production of stevioside and rebaudioside-A in *Stevia rebaudiana* via nutritional and non-nutritional mechanisms. *Applied Soil Ecology*, **72** : 187-194.

Mohammed, N. J. (2016). Response of growth,yield of Tomatillo plant *Physalis pruinosa* L. for inoculation with Mycorrihza and foliar spray with α–Tocoferol and Caroblizer. High Diplomain Agriculture Sciences, Horticulture & Landscape Gardening. College of Agriculture, University of Baghdad 53pp.

Mubarak, M. H., A. H. Belal, I. H. Geddaw and M. I. Nasr (2008). Meeting the challenges of sugar crops and integrated industries in developing countries, Al-Arish, Egypt, pp. 293-298.

Murphy, W. L., D. H. Kohn and D. J. Mooney (2000). Growth of continuous bonelike mineral within porous poly (lactide-co-glycolide) scaffolds *in vitro*. *J. Biomed. Mater. Res*., **50**: 50-58.

Pemba, H. B. and A. B. Sharangi (2016). Stevia :Medicinal Miracles and Therapeutic Magic. *Int. J.of Crop Sci.and Technol*., **2(2)**:45-59.

Rafiee H., H. Naghdi, A. Mehrafarin, A. N. Zarinpanjeh, A. Sekara and E. Zand (2016). Application of plant biostimulants as new approach to improve the biological responses of medicinal plants- A Critical Review. *Journal of Medicinal Plants*, **15(59)**:1-34.

Rai, M., D. Acharya, A. Singh and A. Varma (2001). Positive growth responses of the medicinal plants Spilanthes calva and Withanio somnifera to inoculation by *Piriformospra indica* in a field trail. *Mycorrhiza*, **11**: 123-128.

Salachna, P., A. Byczyñska, I. Jeziorska and E. Udycz (2017). Plant growth of *Verbena bonariensis* L. after chitosan, gellan gum or iota-carrageenan foliar applications. *World Scientific News*, **62** : 111-123.

Salama, A. M, E. A. Ahmed and M. S. Negm (2016). Influence of Foliar Application with some Bio-Stimulants on Growth, Yield, Anatomical Structure and Chemical Composition of Stevia Plant (*Stevia rebaudiana* Bertoni). *Middle East Journal of Agriculture Research*, **5(1)** : 50-63.

Santosh, D. T., K. N. Tiwari and R. G. Reddy (2017). Banana Bunch Covers for Quality Banana Production-A Review. *Int. J. Curr. Microbiol.App. Sci*., **6(7)**: 1275-1291.

Saif -Eldeen, U. M., M. M. B. Shokr and R. S. EL Shotoury (2014). Effect of foliar spray with seaweeds extract and chitozan on earliness and productivity of globe artichoke. *J. Plant Production, Mansoura Univ*., **5 (7)**: 1197-1207.

Singh, A., J. Sharma, K. H. Rexer and A.Varma (2000). Plant productivity determinants beyond minerals, water and light, piriformospora indica: a revolutionary plant growth promoting fungus. *Current Science*, **79**: 1548-1554.

Smith, S. E. and D. W. Read (1997). *Mycorrhizal symbiosis*, 2nd ed., Academic, London.

Suhail, F. M. (2013). Effect of mycorrhizal fungi inoculation and seaweed extract spray on some growth characters and yield of cucumber (*Cucumis sativus* L.). *Journal of Genetic and Environmental Resources Conservation*, **1(3)** : 209-214.

Taleie, N., Y. Hamidoghli, B. Rabiei and S. Hamidoghli (2012). Effects of plant density and transplanting date on herbage,stevioside, phenol and flavonoid yield of *Stevia rebaudiana* Bertoni. *International Journal of Agriculture and Crop Sciences*, **4 (6)** : 298-302.

Varma, A., A. Singh, N. Sudha, S. Sahay, J. Sharma, A. Roy, M. Kumari, D. Rana, S. Thakran, D. Deka, K. Bharti, P. Franken, T. Hurek, O. Blechert, K. H. Rexer, G. Kost, A. Hahn, B. Hock, W. Maier, M. Walter, D. Strack and I. Kranner (2001). *Piriformospora indica*: a cultivable mycorrhiza-like endosymbiotic fungus. Mycota IX, Springer Series, Germany, pp: 123-150.

Vafadar, F., R. Amooaghaie and M. Otroshy (2014). Effects of plant-growth-promoting rhizobacteria and arbuscular mycorrhizal fungus on plant growth, stevioside, NPK and chlorophyll content of Stevia rebaudiana. *Journal of Plant Interactions*, **9(1)**:128-136.

Vanìk, T., A. Nepovim and P. Valicek (2001). Determination of Stevioside in Plant Material and Fruit Teas. *Journal of Food Composition and Analysis*, **14(4)**: 383-388.

Varanuj, C. and M. Chatchai (2009). Stevioside and related compounds: Therapeutic benefits beyond sweetness. *Pharmacology and Therapeutics Journal*, **121** : 41–54.

Vestberg, M. and A. C. Cassells (2009). The use of AMF and PGPR inoculants singly and combined to promote micro-plant establishment growth and health .In: Varma, A. and A. C. Kharkinal (eds.). *Symbiotic fungi. principles and practice*, Springer-Verlay p.337-364.

Waller, F., B. Achatz, H. Baltruschat and J. Fodor (2005). The endophytic fungus *Piriformospora indica* reprograms barley to salt-stress tolerance, disease resistance and higher yield. *Proceedings of the national Academy of Sciences of United State of America*, **38** : 13386-13391.

Wu, Q. S. and R. X. Xia (2006). Arbuscular mycorrhizal fungi influence growth, osmotic adjustment and photosynthesis of citrus under well-watered and water stress conditions. *Journal of Plant Physiology*, **163**: 417- 425.