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EsamAbd-ElkareemAbd-ElazeemAl-Azzony
Medicinal and Aromatic Plants
Research Department,
Horticulture Research
Institute, Agriculture Research
Center, Giza, Egypt

TamerFarouk AhmedEl-Moghazy
Medicinal and Aromatic Plants
Research Department,
Horticulture Research
Institute, Agriculture Research
Center, Giza, Egypt

Effect of magnesium, iron and carbon dioxide on Quality of *Stevia rebaudiana* (Bertoni) Plants

EsamAbd-ElkareemAbd-ElazeemAl-Azzony and TamerFarouk AhmedEl-Moghazy

Abstract

The present research was conducted during 2015-2016 seasons to study the effect of magnesium, iron and carbon dioxide on vegetative growth, roots and quality parameters; the reducing sugars %, non-reducing sugars %, total sugars and Stevioside content of *Stevia rebaudiana* (Bertoni). The treatments were: control; 0Mg:0Fe:0CO₂, T2: 0Mg:0Fe:1 CO₂, T3: 0Mg:0Fe:2 CO₂, T4:1Mg:1Fe:0 CO₂, T5; 1Mg:1Fe:1 CO₂, T6; 1Mg:1Fe:2 CO₂, T7; 1Mg:2Fe: 0 CO₂, T8; 1Mg: 2Fe: 1 CO₂, T9; 1Mg:2Fe: 2 CO₂, T10; 2Mg:1Fe: 0 CO₂ and T11; 2Mg:1Fe: 1 CO₂ as well as T12; 2Mg:1Fe: 2 CO₂. Stevioside content was determined by (HPLS). Results revealed that all combinations (fertilizing treatments) of magnesium, iron and carbon dioxide achieved the highest vegetative growth, roots, reducing sugars %, non-reducing sugars % and total sugars as well as stevioside content compared to control. Moreover, fertilizing treatments 1Mg: 1Fe:1 CO₂ and 1Mg: 1Fe:2 CO₂ achieved average values, while treatments; 2Mg: 1Fe: 2 CO₂, 2Mg: 1Fe: 1 CO₂ and 1Mg: 2Fe: 2 CO₂, respectively recorded higher values for the vegetative growth, roots and quality properties of *Stevia rebaudiana* (Bertoni) during this study than control and other fertilizing treatments.

Keywords: *Stevia rebaudiana* (Bertoni), magnesium, iron, carbon dioxide

1. Introduction

Stevia rebaudiana (Bertoni) is a perennial herb of the Asteraceae family native to certain region of South-America (Brazil and Paraguay). It is often referred as "The sweet herb of Paraguay". The leaves of stevia contain active principle stevioside which, 200-300 times sweeter than sugar [1]. Stevia is likely to become a major source of high potency sweetener for the growing natural food market in the future. The task hand is to convert stevia from a wild plant to modern crop well suited to efficient mechanized production [2]. Uses of Stevia (safe for diabetics, it does not affect blood sugar levels, does not have the neurological or renal side effects as other artificial sweeteners, possess anti-fungal and anti-bacterial properties, It can be safely used in herbal medicines, tonics for diabetic patients and also in daily usage products such as mouthwashes and toothpastes, mild Stevia leaf tea offers excellent relief for an upset stomach and its white crystalline compound (Stevioside) is the natural herbal sweetener with no calories) [3]. Also has been used to help control weight in obese persons [4]. Stevia plant and its extract both are used in weight-loss programmes because of their ability to reduce the craving for sweet and fatty foods [5]. Glycosides responsible for the plants sweeteners were discovered in 1931. Eight of stevia glycosides were discovered, viz. dulcosides A, rebaudiosides A- E, steviobioside and stevioside [6]. Magnesium (Mg²⁺) plays a role in enzyme activation, sugar synthesis, oil and fat formation and control of nutrient uptake, as well as other processes [7]. Mg²⁺ is also a very important in both the light and dark reactions of photosynthesis as it is the central atom in the chlorophyll molecule and as a metallic cofactor in many enzymes including ATP ases, protein kinases and Rubisco [8, 9]. Shortage of Mg²⁺ leads to the loss of color between the veins in the leaves with the veins themselves staying green. furthermore, any plants with insufficient amounts of Mg²⁺ shows lower ability to perform photosynthesis and become susceptible to photo-oxidative damage [7]. In leaves, Mg²⁺deficiency suppressed carbon dioxide (CO₂) uptake with corresponding decreases in CO₂ fixation, it also inhibited sucrose export from leaves, whereas enhanced production of reactive oxygen species [10]. Iron (Fe) is a necessity micronutrient for almost all living organisms because of it plays critical role in metabolic processes such as DNA synthesis, photosynthesis and respiration. Fe plays a big role in various physiological and bio-chemical pathways in plants. It serves as a constituent of much vital enzymes such as cytochromes of the electron transport chain, and it is thus required for a wide range of biological functions. In plants, Fe is participated in the synthesis of chlorophyll, and it is necessity for the maintenance

Corresponding Author:
Swati Chobhe
EsamAbd-ElkareemAbd-ElazeemAl-Azzony
Medicinal and Aromatic Plants
Research Department,
Horticulture Research
Institute, Agriculture Research
Center, Giza, Egypt

of chloroplast structure and function^[11]. Fe is the fourth most abundant element on earth, and soil typically contains 1–5% total iron. Most Fe in the soil is found in iron oxides, silicate minerals and hydroxides forms that are not readily available for plant use^[12].

Increment in the present atmospheric carbon dioxide concentration foresees for the end of this century will increase crop growth, development and grain yield^[13, 16]. Elevated atmospheric carbon dioxide (eCO₂) increase the yield of vegetables and could also affect their nutritional quality,^[17] conducted a meta-analysis using 57 articles consisting of 1,015 observations and found that eCO₂ increased the concentrations of fructose, total soluble sugar, glucose, total phenols, total antioxidant capacity, total flavonoids, ascorbic acid, and calcium in the edible part of vegetables. The atmospheric CO₂ concentration has increased from 280 μmol mol⁻¹ before the industrial revolution to 408 μmol mol⁻¹ in March 2018) mainly due to fossil fuel combustion and deforestation. It is predicted to reach 1000 μmol mol⁻¹ by the end of this century^[18]. Elevated eCO₂ can promote net photosynthetic rates of plants and thus plant productivity and yield^[19]. It also enhances plant tolerance to environmental stresses via increased antioxidants and soluble sugars as well as root exudates^[20, 21]. Therefore, eCO₂ has been widely used as a gas fertilizer in greenhouse vegetables cultivation, particularly in recent decades as greenhouse technologies have improved^[22, 23] and the demand for vegetables is continuously increasing. The target from the current investigation is to study the impact of using combinations of magnesium, iron and carbon dioxide on improving the growth and productivity of *Stevia rebaudiana* (Bertoni) plants.

2. Materials and Methods

2.1 Plant material

The study was carried out at the Faculty of Agriculture, Suez

Canal University, Egypt during two successive seasons of 2015/2016 to study the effect of magnesium, iron and CO₂ on *Stevia rebaudiana* (Bertoni). The *Stevia* seedlings were planted at the first of April in each season. CO₂ was sprayed four times, the first time was after planted 2 weeks then each 15 days in each cut for both seasons the same times for magnesium and iron (add with irrigation water). The unit from magnesium 1gm/L, iron 0.5 gm/L, and CO₂ 50, 75 gm/100L water. The source of CO₂ treatment was Fertilizer; LITHOVIT that produced from Ziovita Company (Germany), which contains calcium (3%, source Calcium chloride) and magnesium (2%, source Magnesium chloride) in addition to carbonate calcium (24%) and magnesium carbonate (41%). It has the ability to enter directly through the respiratory vents and edit to active CO₂ for photosynthesis. While the source of magnesium and iron were (magnesium sulphate; 16% mg, 12% S and iron sulphate; 19% Fe, 11% S, Respectively). The chemical analysis of the used soil and irrigation water are presented in Table (1) according to the method described by^[24].

2.2 The treatments were conducted as follows

- 1- Control (0Mg: 0Fe: 0 CO₂).
- 2- 0Mg: 0Fe: 1 CO₂
- 3- (0Mg: 0Fe: 2 CO₂)
- 4- (1Mg: 1Fe: 0CO₂
- 5- 1Mg: 1Fe: 1 CO₂
- 6- 1Mg: 1Fe: 2 CO₂
- 7- 1Mg: 2Fe: 0CO₂
- 8- 1Mg: 2Fe: 1CO₂
- 9- 1Mg: 2Fe: 2CO₂
- 10- 2Mg: 1Fe: 0 CO₂
- 11- 2Mg: 1Fe: 1CO₂
- 12- 2Mg: 1Fe: 2 CO₂

Table 1: The chemical analysis of the used soil and irrigation water

Particle size distribution %		Soluble cations (mmol.l ⁻¹)				Soluble anions (mmol.l ⁻¹)			
Sand	92.8	K ⁺		1.83	CO ₃ ²⁻		---		
Silt	4.00	Na ⁺		4.30	HCO ₃		4.67		
Clay	1.76	Mg ⁺²		3.86	CL ⁻		10.72		
Textural class	sand	Ca ⁺²		4.89	SO ₄ ⁺²		5.00		
PH (1;2.5)	6.84					N (mg/kg)		7.79	
EC (dSm ⁻¹)	2.25					P (mg/kg)		6.81	
						K (mg/kg)		47	
irrigation water									
EC dSm ⁻¹	PH	Cations (meq/l)				Anions (meq/l)			
0.408	7.89	Ca ²⁺	Mg ²⁺	Na ⁺	K ⁺	CO ₃ ²⁻	HCO ₃	CL ⁻	SO ₄ ²⁺
		1.67	1.31	1.01	0.24	---	2.89	0.89	0.11

2.3 Harvest

Stevia plants were harvested two times in each season, the first cut was in July^{1st} while the second cut was in October^{1st} bin both seasons. Then the following data were recorded

Plant height (cm)

Number of branches plant

Fresh and dry weight (g/plant.)

Reducing sugars content, non-reducing sugars content and total sugars content in *stevia* leaves were determined according to the procedure described by^[25]. Stvioside content in the dried leaves was assessed according to the method defined by^[26]. The developed procedure involves two steps: solvent extraction followed by an isocratic HPLS analysis., 1 g of dried leaves of *Stevia rebaudiana*, is ground and solvent-

extracted with 100 ml EtOH 70% (W/W) in 250 ml Erlenmeyer flasks by shaking for 30 min in a 70 degrees C water bath. After the extract was cooled, a 10 ml aliquot it was filtered, then 5ul was analyzed by HPLC. Quantitation was performed by means of an external standard calibration curve for each analyte which had been obtained from standard solutions of pure stevioside and rebaudioside A.

2.4 Experimental design and statistical analysis

Data of the present study were subjected to the analysis of variance test (ANOVA) as Randomized Complete Blocks Design. The least significant differences (LSD) at the 5% level of probability were calculated using a computer program (Costat, version 6.303, 2005) according to^[27].

3. Results and Discussion

3.1 Results

3.1.1 Effect of magnesium, iron and carbon dioxide on plant height (cm) and number of branches/plants

All fertilizing treatments led to a sharp increase in *Stevia rebaudiana* (Bertoni) plant height and number of branches /plant compared to control treatments during the 2015/2016

seasons Table (2) and Fig. (1). In addition, fertilizing treatments (1Mg: 2Fe:1CO₂, 1Mg: 2Fe: 2CO₂, 2Mg: 1Fe: 0 CO₂, 2Mg: 1Fe: 1CO₂, 2Mg: 1Fe: 2CO₂) recorded a marked increase in plant height and number of branches / plan than other treatments. Treated plants with 2Mg: 1Fe:1CO₂ and 2Mg: 1Fe:2CO₂ were the highest values than other fertilizing treatments.

Table 2: Effect of magnesium, iron and carbon dioxide on Plant hight (cm) and number of branches of *Stevia rebaudiana* (Bertoni) during 2015/2016 seasons.

Treatments	First season						Second season					
	Plant height(cm)			branches No			Plant height (cm)			branches No		
	1 st Cut	2 nd Cut	Mean	1 st Cut	2 nd Cut	Mean	1 st Cut	2 nd Cut	Mean	1 st Cut	2 nd Cut	Mean
0Mg: 0Fe: 0 CO ₂	34.33	28.66	31.50	7.00	10.66	8.83	32.33	23.66	28.00	6.33	10.33	8.33
0Mg: 0 Fe:1 CO ₂	38.00	27.66	32.83	9.33	17.00	13.16	32.66	24.00	28.33	7.66	12.66	10.16
0Mg: 0 Fe:2 CO ₂	43.00	30.33	36.66	12.66	19.00	15.83	35.66	26.66	31.16	9.66	14.33	12.00
1Mg: 1Fe: 0 CO ₂	47.66	35.33	41.50	15.00	21.66	18.33	42.00	29.66	35.83	13.33	20.66	17.00
1Mg:1 Fe:1 CO ₂	53.00	39.33	46.16	16.66	23.33	19.99	47.00	34.00	40.5	13.00	20.33	16.66
1Mg:1 Fe:2 CO ₂	57.00	44.33	50.66	17.66	26.66	22.16	52.66	39.00	45.83	15.66	24.00	19.83
1Mg: 2 Fe: 0CO ₂	61.00	46.33	53.66	18.66	28.66	23.66	54.00	39.66	46.83	15.66	25.33	20.50
1Mg:2 Fe:1 CO ₂	64.00	48.00	56.00	20.33	31.66	26.00	56.33	42.33	49.33	18.66	28.00	23.33
1Mg:2 Fe:2 CO ₂	64.00	50.66	57.33	21.66	32.00	26.83	58.00	43.00	50.50	18.66	28.66	23.66
2Mg:1 Fe: 0 CO ₂	63.00	49.66	56.33	19.66	29.66	24.66	57.00	43.33	50.16	17.33	27.33	22.33
2Mg:1 Fe:1 CO ₂	66.66	50.33	58.50	24.66	36.33	30.50	58.66	44.66	51.66	21.33	33.00	27.16
2Mg:1 Fe:2 CO ₂	68.66	53.66	61.16	25.33	39.33	32.33	61.00	44.33	52.66	24.00	36.33	30.16
Mean	55.02	42.02		17.38	26.33		48.94	36.19		15.11	23.41	
LSD 0.05 Treatments	3.069			2.03			2.601			2.247		
Cuts	1.253			0.829			1.0619			0.917		
Inte. treatments x cuts	3.623			2.395			3.070			2.638		

Regarding the effect of 1st and 2nd cut, results the first recorded in first cut of both seasons gave significantly higher plants than 2nd cut. On the other side branches number increased significantly in 2nd cut than 1st cut in both seasons.

number was lower in the first cut than the 2nd cut in all treatments in both seasons.

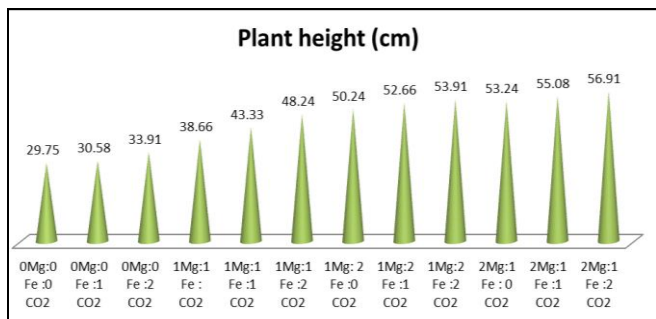


Fig 1: Effect of magnesium, iron and carbon dioxide on Plant height (cm) in both seasons.

3.1.2 Effect of magnesium, iron and carbon dioxide on fresh and dry weights (g. /plant)

Data offered in Table (3) showed that effect of magnesium, iron and carbon dioxide on fresh and dry weights (g. /plant) of *Stevia rebaudiana* (Bertoni) during 2015/2016 seasons. The untreated plants (0Mg: 0Fe: 0CO₂) recorded the lowest values for fresh and dry weight (g. /plant) in two seasons when compared with treated plants. The treatments (1 Mg: 2 Fe: 2CO₂, 2 Mg: 1 Fe: 1CO₂, 2 Mg: 1 Fe: 2CO₂) were registered better values than other treatments. Also 2Mg: 1Fe:2 CO₂ fertilizing treatment was the greatest on increased fresh and dry weight (g. /plant) in the present study.

Evaluating the interaction effect between treatments and the tested cuts, data showed that the interactions registered the lowest numbers for plant height (cm) with second cut in both seasons in all fertilizing treatments. While the branches-

Regarding the impact of 1st and 2nd cut, results obviously showed that fresh and dry weight (g. /plant) were higher in second cut of the first and second seasons than in 1st cut for both seasons.

The interaction influence between treatments x tested cuts, the obtained results indicated that the interactions registered the lowest values of fresh and dry weight (g. /plant) with the first cut in both seasons under fertilizing treatments.

Table 3: Effect of magnesium, iron and carbon dioxide on fresh weight (g /plant) and dry weight (g /plant) of *Stevia rebaudiana* (Bertoni) during 2015/2016 seasons

Treatments	First season						Second season					
	Fresh weight (g./plant)			Dry weight (g./plant)			Fresh weight (g./plant)			Dry weight (g./plant)		
	1 st Cut	2 nd Cut	Mean	1 st Cut	2 nd Cut	Mean	1 st Cut	2 nd Cut	Mean	1 st Cut	2 nd Cut	Mean
0Mg: 0Fe: 0 CO ₂	51.40	55.53	53.46	11.06	12.33	11.70	41.66	44.86	43.26	9.00	10.00	9.50
0Mg: 0 Fe:1 CO ₂	58.73	63.46	61.10	13.46	14.86	14.16	49.13	53.40	51.26	11.13	12.53	11.83
0Mg: 0 Fe:2 CO ₂	71.00	77.20	74.10	16.46	17.73	17.10	60.60	66.46	63.53	13.60	15.20	14.40
1Mg:1 Fe: 0CO ₂	88.33	95.86	92.10	18.93	21.06	20.00	69.00	76.00	73.40	16.40	18.20	17.30
1Mg:1 Fe: 1 CO ₂	103.80	113.00	108.40	21.73	24.06	22.90	83.80	91.86	87.83	20.60	22.80	21.70
1Mg:1 Fe: 2 CO ₂	110.60	120.06	115.33	25.73	27.86	26.80	99.13	109.53	104.26	25.53	27.86	26.70
1Mg: 2 Fe: 0 CO ₂	134.93	146.93	140.93	32.40	35.20	33.80	109.93	119.73	114.83	26.53	29.33	27.93

1Mg:2 Fe:1 CO ₂	156.13	173.73	164.93	35.06	37.93	36.50	129.20	140.93	135.06	30.40	33.40	31.90
1Mg:2 Fe:2 CO ₂	185.26	204.53	194.90	41.40	45.06	43.23	149.20	164.53	156.86	37.00	40.40	38.70
2Mg:1 Fe: 0 CO ₂	137.73	151.73	144.73	28.33	30.26	29.30	134.20	146.66	140.43	31.00	34.20	32.60
2Mg:1 Fe:1 CO ₂	205.60	227.60	216.60	45.13	49.20	47.16	164.60	179.66	172.03	38.80	42.40	40.60
2Mg:1 Fe:2 CO ₂	219.46	243.20	231.33	49.06	53.40	51.23	185.46	204.26	194.86	43.20	46.73	44.96
mean	126.91	139.40		28.23	30.75		106.33	116.50		25.26	27.75	
LSD 0.05 Treatments	9.59			2.366			18.52			4.99		
Cuts	3.915			0.966			7.56			2.037		
Inte. treatments x cuts	11.31			2.791			21.861			5.89		

3.1.3 Effect of magnesium, iron and carbon dioxide on root length

The obtained results revealed that all fertilizing treatments during the both seasons had enhanced the root length of *Stevia rebaudiana* (Bertoni) plants than 0mg: 0 Fe: 0CO₂treatment (control) Table (4). However 2Mg: 1Fe:1 CO₂, 2Mg: 1Fe:2 CO₂ and 1Mg: 2Fe:2 CO₂ treatments created clear enhancement on root length than the other fertilizing treatments. Meanwhile treatments (1Mg: 2Fe:0 CO₂ and 1Mg: 1Fe:2CO₂) recorded average values in both seasons on root length.

Table 4: Effect of magnesium, iron and carbon dioxide on root length of *Stevia rebaudiana* (Bertoni) during 2015/2016 seasons.

Treatments	First season	Second season
	Root length	Root length
0Mg: 0Fe: 0 CO ₂	9.83	9.16
0Mg: 0 Fe: 1 CO ₂	11.16	10.83
0Mg: 0 Fe: 2 CO ₂	13.50	12.83
1Mg:1 Fe: 0CO ₂	14.33	13.83
1Mg:1 Fe: 1 CO ₂	14.66	14.16
1Mg:1 Fe: 2 CO ₂	14.83	13.33
1Mg: 2 Fe: 0 CO ₂	15.16	14.83
1Mg:2 Fe: 1 CO ₂	17.33	16.33
1Mg:2 Fe: 2 CO ₂	18.16	17.16
2Mg:1 Fe: 0 CO ₂	16.50	15.00
2Mg:1 Fe: 1 CO ₂	20.16	18.50
2Mg:1 Fe: 2 CO ₂	21.16	19.50
LSD 0.05	1.21	1.32

3.1.4. Effect of magnesium, iron and carbon dioxide on reducing sugars %, non-reducing sugars % and total sugars %

The results concerning the influence of magnesium, iron and

carbon dioxide on reducing sugars %, non-reducing sugars % and total sugars of *Stevia rebaudiana* (Bertoni) during 2016 season, showed that the treated plants (fertilizing treatments) significantly increased reducing sugars %, non-reducing sugars % and total sugars% in second seasons when compared with untreated plants Table (5) and Fig. (2). Also the results showed that the best fertilizing treatments 2Mg: 1Fe:1CO₂ and 2Mg: 1Fe: 2CO₂ than control and other fertilizing treatments. Furthermore 1Mg: 2Fe:0CO₂ treatment recorded medium value 5.09 % from reducing sugars % while 1Mg: 1Fe:0CO₂ treatment gave the same result for non-reducing sugars % and total sugars%.

Table 5: Effect of magnesium, iron and carbon dioxide on reducing s %, non-reducing s % and total S% of *Stevia rebaudiana* (Bertoni) during 2016 season

Treatments	Second season		
	Reducing s %	Non-reducing s%	Total S%
0Mg: 0 Fe: 0 CO ₂	2.55	7.73	10.80
0Mg: 0 Fe: 1 CO ₂	3.08	8.52	11.79
0Mg: 0 Fe: 2 CO ₂	3.24	8.97	13.15
1Mg:1 Fe: 0 CO ₂	3.82	9.42	14.31
1Mg:1 Fe: 1 CO ₂	4.22	9.89	15.17
1Mg:1 Fe: 2 CO ₂	4.65	10.28	15.69
1Mg: 2 Fe: 0 CO ₂	5.09	10.80	16.36
1Mg:2 Fe: 1 CO ₂	6.09	11.14	17.13
1Mg:2 Fe: 2 CO ₂	6.42	11.35	17.85
2Mg:1 Fe: 0 CO ₂	5.61	11.06	16.82
2Mg:1 Fe: 1 CO ₂	6.86	11.89	18.36
2Mg:1 Fe: 2 CO ₂	7.13	12.15	18.86
LSD 0.05	0.186	0.297	0.504

Total sugars %

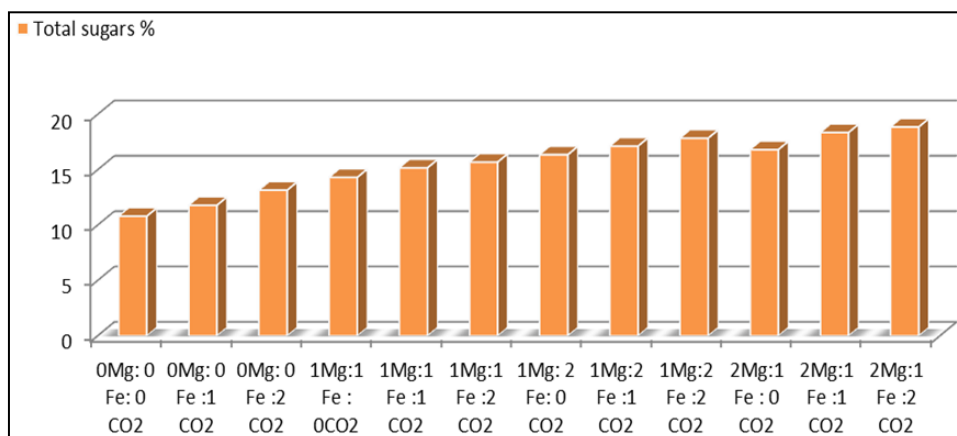


Fig 2: Effect of magnesium, iron and carbon dioxide on total sugars %.

3.1.5. Effect of magnesium, iron and carbon dioxide on Stevioside %

Data presented in Table (6) and Fig. (3) Showed that HPLC analysis of the Stevioside % from *Stevia rebaudiana* (Bertoni) plants during 2016 season. The result declared that all tested

fertilizing treatments increased Stevioside % when compared with control. However, 1Mg: 2Fe: 2CO₂ and 2Mg: 1Fe: 1CO₂ as well as 2Mg: 1Fe: 2 CO₂ treatments recorded great values than other treatments and control. While treatments 1Mg: 2Fe: 1CO₂ and 2Mg: 1Fe: 0 CO₂gavemedium values 9.36 and

9.16 respectively from Stevioside %.

Table 6: Effect of magnesium, iron and carbon dioxide Stevioside% of *Stevia rebaudiana* (Bertoni) during 2016 season.

Treatments	Stevioside %
0Mg: 0Fe: 0 CO ₂	8.76
1Mg:2 Fe:1 CO ₂	9.36
1Mg:2 Fe:2 CO ₂	9.76
2Mg:1 Fe: 0 CO ₂	9.16
2Mg:1 Fe:1 CO ₂	10.12
2Mg:1 Fe:2 CO ₂	10.98

Stevioside %

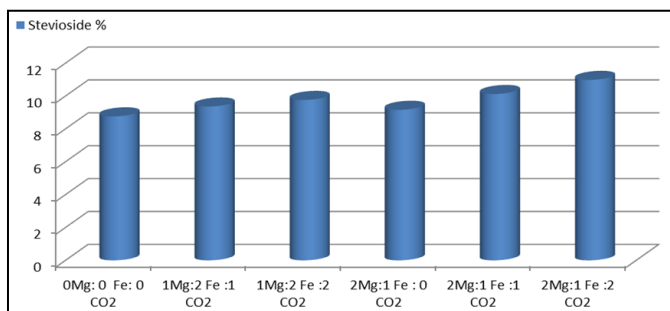


Fig 3: Effect of magnesium, iron and carbon dioxide on Stevioside %

3.2 Discussion

3.2.1 Vegetative growth

In the present experiment, the obtained results showed that all combinations (fertilizing treatments) of magnesium and iron as well as carbon dioxide enhanced vegetative growth and roots. These results may be due to magnesium (Mg+2) is a very important in both the dark and light reactions of photosynthesis as it is the central atom in the chlorophyll molecule and as a metallic cofactor in many enzymes including ATP ases, [8, 9]. These results are in agreement with those obtained by [28] found that the most effective treatment for enhancing the plant stems/leaves and fresh and dry weights in almost 3 cuts, as well as fresh and dry yield per feddan gave the highest values with magnesium level (75 kg/feddan) during both seasons in all cultivars parsley (Petra, Krausa and Bravour). These results are in conformity with those obtained by [29] on thyme plants. And also found that the magnesium fertilization plays an important role in increase of productivity, crop protection and green color [30, 36]. Improvement of plant growth in response to magnesium usage was demonstrated previously by [37, 38]. Identical results were obtained by [39] reported that plants receiving magnesium might have been helped in terms of vigorous root growth, fashioning of chlorophyll, resulting in higher photosynthesis and protein content, which might have resulted in better growth and higher dry matter production.

Moreover, Positive effect of iron on vegetative growth of *Stevia rebaudiana* (Bertoni) plants may be due to Fe plays critical role in metabolic processes such as DNA synthesis, photosynthesis and respiration. It serves as a component of much vital enzymes such as cytochromes of the electron transport chain. In plants, Fe is participated in the synthesis of chlorophyll, and it is necessity for the maintenance of chloroplast structure and function [11]. These results are in harmony with those reported by [40] found that Fe had beneficial effect on yield and volatile oil production of chamomile plant. [41] Reported that low levels of iron and Mn were essential for better plant growth and efficient utilization of these nutrients by rice plants. The obtained results showed

that carbon dioxide increased vegetative growth. These results are in agreement with those obtained by [42] reported that elevated carbon dioxide has an ameliorative impact on the growth of Alfalfa with increment performance in parameters related to production function of the plant. Also elevated carbon dioxide (eCO₂) can promote net photosynthetic rates of plants and thus plant productivity and yield [19]. Therefore, eCO₂ has been widely used as a gas fertilizer in greenhouse vegetable cultivation, particularly in recent decades as greenhouse technologies have improved [22, 23]. These results are in line with those stated by [17] found that elevated atmospheric CO₂ (eCO₂) increased the yield of vegetables.

3.2.2 Quality parameters

The obtained results indicate that all combinations (fertilizing treatments) of magnesium, iron and carbon dioxide increased the quality parameters; reducing sugars %, non-reducing sugars % and total sugars% as well as Stevioside %. These results may be due to magnesium (Mg+2) plays a part in enzyme activation, sugar synthesis, control of nutrient uptake, and other processes, [7]. Moreover, Positive effect of iron on the quality parameters of *Stevia rebaudiana* (Bertoni) plants may be due to Fe plays a big role in various physiological and bio-chemical pathways. It is thus required for a wide range of biological functions in plants [11]. These results are in harmony with those reported by [40] found that Fe had beneficial effect on yield and volatile oil production of chamomile plant. Iron is a cofactor for a great number of enzymes that catalyze many bio-chemical processes within the plant [43, 44]. The results revealed that carbon dioxide enhanced the quality parameters; reducing sugars %, non-reducing sugars %, total sugars % and Stevioside %. These results are in line with those stated by [17] found that elevated atmospheric CO₂ (eCO₂) increased the concentrations of fructose, total soluble sugar, glucose, total antioxidant capacity, total flavonoids, total phenols, ascorbic acid, and calcium in the edible part of vegetables.

4. Conclusion

Results revealed that all combinations of magnesium, iron and carbon dioxide achieved the highest vegetative growth, roots, reducing sugars %, non-reducing sugars % and total sugars as well as stvioside content compared to control. Moreover, fertilizing treatments; 2Mg: 1Fe:1CO₂, 2Mg: 1Fe:2CO₂ and 1Mg: 2Fe:2CO₂ recorded higher values for quality parameters of *Stevia rebaudiana* (Bertoni) during this study than control and other fertilizing treatments.

5. References

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