

## ANTIOXIDANTS ACTIVITY IN STEVIA PLANT AS EFFECTED BY SHADING AND FOLIAR SPRAYING OF MORINGA AND LIQUORICE EXTRACT

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### ABSTRACT

A field experiment was conducted in the fields of the College of Agricultural Engineering Sciences/University of Baghdad for the year 2021-2022 to study the effect of shading and the foliar application of some plant extracts on the growth, yield and natural antioxidants activity in the Stevia plant leaves. The experiment was conducted using a randomized complete block design (RCBD) using a split-plot arrangement with three replicates. The results showed that S<sub>1</sub> significantly outperformed the leaves' dry weight by 41.49 g plant<sup>-1</sup> and leaves' dry weight per hectare by 6.88 tons ha<sup>-1</sup>. On the other hand, S<sub>2</sub> showed significant superiority in the total antioxidant activity by 11000.20 mg L<sup>-1</sup>, total phenols by 9.947 mg L<sup>-1</sup>, and leaves the chlorophyll b content by 76.16 mg 100 g<sup>-1</sup>. Furthermore, M<sub>2</sub> significantly outperformed in the dry weight of the leaves with 38.21 g plant<sup>-1</sup>, followed by G<sub>2</sub> with 36.30 g plant<sup>-1</sup>.

Similarly, the dry weight of the leaves per hectare reached 6.33 tons ha<sup>-1</sup> in the M<sub>2</sub>, followed by G<sub>2</sub> with 6.13 tons ha<sup>-1</sup>. G<sub>2</sub> also excelled in total antioxidant activity with 12852.50 mg L<sup>-1</sup>, while M<sub>1</sub> showed significant superiority in total phenols with 16135 mg L<sup>-1</sup>. M<sub>1</sub> also showed a higher chlorophyll concentration with 189.45 mg 100 g<sup>-1</sup>, followed by G<sub>1</sub> with 188.55 mg 100 g<sup>-1</sup> and M<sub>2</sub> with 180.50 mg 100 g<sup>-1</sup>. The liquorice extract (G<sub>2</sub>) exhibited the highest overall antioxidant activity and chlorophyll b when interacting with shaded plants. Additionally, the Moringa extract (M<sub>1</sub>) contributed to a significant increase in total phenols in shady Stevia plants.

Keywords: Stevia plant, Shading, Antioxidants, Plant extracts.

نشاط مضادات الأكسدة في نبات الستيفيا بتأثير التظليل والرش الورقي لمستخلص المورينجا وعرق السوس

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### الخلاصة

زرعت شتلات نبات ورق السكر (الستيفيا) والناجة من الزراعة النسيجية للصف الاسباني Spanti في منطقة الجادرية- بغداد بتاريخ 15-3-2021 بهدف دراسة تأثير دور التظليل وبعض المستخلصات النباتية في نمو وحاصل الاوراق ومحتواها من مضادات الاكسدة الطبيعية في نبات ورق السكر (الستيفيا). نفذت تجربة حقلية في حقول كلية علوم

\*The article is taken from a master's thesis by the first researcher.

الهندسة الزراعية / جامعة بغداد للعام 2021-2022، وفقاً لتصميم القطاعات الكاملة المعشاة (RCBD) ضمن ترتيب الألواح المنشقة بثلاثة مكررات. اظهرت النتائج تفوق  $S_1$  معنوياً في الوزن الجاف للأوراق 41.49 غم نبات<sup>1</sup> والوزن الجاف بالهكتار 6.88 طن ه<sup>1</sup>، أما  $S_2$  فقد تفوق معنوياً في الفعالية الكلية لمضادات الأكسدة 11000.20 ملغم لتر<sup>1</sup> والفينولات الكلية 9947 ملغم لتر<sup>1</sup> ومحتوى الأوراق من كلوروفيل b الذي بلغ 76.16 ملغم 100 غم<sup>1</sup> وزن رطب. كما تفوق  $M_2$  معنوياً في الوزن الجاف للأوراق بلغ 38.21 غم نبات<sup>1</sup> يليه  $G_2$  بلغ 36.30 غم نبات<sup>1</sup> وحاصل الوزن الجاف للأوراق بلغ 6.33 طن ه<sup>1</sup> يليه  $G_2$  بلغ 6.13 طن ه<sup>1</sup>، وتفوق  $G_2$  في الفعالية الكلية لمضادات الأكسدة 12852.50 ملغم لتر<sup>1</sup>، اما الفينولات الكلية فقد تفوق مستخلص  $M_1$  وبلغ 16135 ملغم لتر<sup>1</sup>. كذلك ظهر تفوق  $M_1$  في محتوى الأوراق من كلوروفيل a الذي بلغ 189.45 ملغم 100 غم<sup>1</sup> وزن رطب يليه  $G_1$  بلغ 188.55 ملغم 100 غم<sup>1</sup> وزن رطب و  $M_2$  بلغ 180.50 ملغم 100 غم<sup>1</sup> وزن رطب. حقق رش مستخلص عرق السوس  $G_2$  أعلى فعالية كلية لمضادات الأكسدة ومحتوى الأوراق من كلوروفيل b بالتداخل مع النباتات المظللة، وساهم مستخلص المورينجا  $M_1$  في اعطاء على نسبة للفينولات الكلية لنباتات الستيفيا المظللة.

الكلمات المفتاحية: نبات الستيفيا، التظليل، مضادات الأكسدة، المستخلصات النباتية.

## INTRODUCTION

Sugar leaf (*Stevia rebaudiana bertonii*) is a perennial herbaceous plant that can reach a height of 60-70 cm and sometimes up to 1 m. Stevia belongs to the Asteraceae family. The genus Stevia includes more than 230 species, but only two of them, *Phlebophylla* and *Rebaudiana*, contain the steviol glycosides compound (Lemus-Mondaca *et al.*, 2012). The native habitat of the sugar leaf plant is South America. It was first discovered in Paraguay in 1887 and later spread as a major cash crop to China, Japan, Pakistan, India, and the rest of the world, including Spain and America (Barroso, 2018). Sugar leaf (Stevia) was recently introduced to Iraq and planted for research purposes in the College of Agricultural Engineering fields in 2017, both in shaded plastic houses and in the open field (Abdul-Qader *et al.*, 2022). In the 1960s, a crystalline white compound was extracted from this plant, and it was found that the crystalline compound is 300 times sweeter than sugar without having a negative effect on blood sugar levels (Kondak *et al.*, 2018). Stevia extract can be used in various food industries, including beverages (Al-Hamdani, 2020), making it ideal for use by diabetic patients, individuals with chronic diseases, and healthy individuals in dietary programs to lower blood sugar, cholesterol, and total fat levels (Al-Hamdani, 2019). The medical importance of the sugar leaf plant lies in its containing several natural antioxidants and its medicinal role in patients with high blood pressure, cancer, and its antimicrobial and antifungal properties (Al-Hamdani *et al.*, 2019). Stevia leaves are a source of diterpene glycosides, with stevioside being the main sweetening compound present in Stevia leaves at a percentage of 5-15%, followed by rebaudioside at 3-6%, and other Stevia glycosides exhibit high chemical stability due to their three-dimensional chemical structure, which provides resistance to acid and enzymatic degradation, ensuring their stability in terms of biochemical and physiological aspects (Mishra *et al.*, 2010). Stevia leaves also contain other compounds such as phenols, flavonoids, terpenes, coumarins, saponins, quinones, and other oils (Al-Hamdani, 2020; Al-Taweel *et al.*, 2022). Due to its sweetness, nutritional value, and the medicinal compounds present in Stevia plant, it has become an important and essential crop soon (Al-Taweel *et al.*, 2021), and the total global trade of Stevia sweeteners exceeded \$771 million in 2022 (Schieber, 2017). Plant extracts have been successfully used as organic fertilizers due to their containing biologically active compounds that stimulate plant growth and improve nutritional



status without harmful effects on many crops (Hoque *et al.*, 2021). Moreover, Al-Temimi & Al-Hilfy (2022) indicated that plant extracts contain natural growth regulators such as auxins, gibberellins, and cytokinins. The study aims to investigate the role of shading as a primary factor and adding aqueous extracts of Moringa leaves and Licorice root extract as a secondary factor in improving growth, yield of dry leaves, and their content of natural antioxidants.

## MATERIALS AND METHODS

A field experiment was conducted in the fields of the College of Agricultural Engineering Sciences / University of Baghdad in the spring season of 2021. Randomized Complete Bock Design (RCBD) with three replications was used using split plot arrangement. The experiment included two factors, the main plots included two levels of shading, the first without shading (exposed) and the second 50% shading and they are denoted S<sub>1</sub> and S<sub>2</sub>, respectively, while the s sub-plots included the spraying of aqueous plant extracts of Moringa at a rate 10 and 15% (Yasmeen *et al.*, 2013), denoted M<sub>1</sub> and M<sub>2</sub> respectively, and licorice extract at a rate 3 and 6% (Hussein, 2002), denoted G<sub>1</sub> and G<sub>2</sub> respectively with an interval of 15 days between the time of each spray, in addition to the M<sub>0</sub>G<sub>0</sub> as control treatment which sprayed with distilled water in three sprays.

The experimental units were distributed with a total of 30 experimental units resulting from the experimental factors (2 × 5 × 3), with 10 experimental units for each replication. Each experimental unit consisted of 18 plants. The plants were cultivated with a spacing of 20 cm between each plant and 30 cm between each row (Abdul-Qader *et al.*, 2022). When the plant reached a height of approximately 60 cm after 60 days of cultivation, the plant was stuffed and the chlorophyll rate (a, b and total) was calculated from the fresh leaves. Also, the leaf yield was collected for drying. The leaves were placed on a table at room temperature ranging from 25-30°C. and the leaves were stirred daily until they reached a stable weight and completely dried (Al Amrani *et al.*, 2018)

## CHARACTERS UNDER STUDY

1. **Dry weight of plant leaves (g plant<sup>-1</sup>):** Selected plants were naturally dried in a special ventilated room with daily turning until reaching a constant weight and complete leaf dryness, then the leaves were weighed and the means was calculated.
2. **Dry leaf yield (ton ha<sup>-1</sup>):** It was calculated from the following equation:  

$$\text{Dry leaf yield (ton ha}^{-1}\text{)} = \text{Dry leaf weight (g plant}^{-1}\text{)} \times \text{Plant density (plants ha}^{-1}\text{)} / 10^6$$
3. **Total antioxidant activity (mg L<sup>-1</sup>):** The antioxidant capacity of the leaves was estimated based on the equivalent of ascorbic acid using the spectrophotometric method according to the method of (Prieto, 1999).
4. **Total phenols (mg L<sup>-1</sup>):** The total phenols in Stevia plant leaves were estimated based on the gallic acid equivalent using a spectrophotometer according to the method described by (Singleton *et al.*, 1999).
5. **Chlorophyll content in leaves (mg 100 g<sup>-1</sup> fresh weight):** Chlorophyll (a, b and total) was calculated using the developed method by Goodwin (1976) and the following equations:  

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



Chlorophyll b (mg L<sup>-1</sup>) = 22.9 D (645) - 4.68 D (663)

Total chlorophyll (mg L<sup>-1</sup>) = 20.2 D (645) + 8.02 D (663)

The results were converted to mg 100g<sup>-1</sup> fresh weight by the following equation:

mg 100 g<sup>-1</sup> fresh weight = mg L<sup>-1</sup> x final volume of extract (L) x 100 / sample weight (g)

The data were statistically analyzed using the Genstat program, and the least significant difference (LSD) test at 0.05 probability level was used to compare between means (Steel & Torrie, 1980).

## RESULTS AND DISCUSSION

### Leaf dry weight (g plant<sup>-1</sup>)

The results reveal significant differences between the studied factors and significant interaction between two factors in the dry leaf weight of the plant (Table 1). The results demonstrate a significant decrease in the mean of S<sub>2</sub> compared to S<sub>1</sub>, with a percentage decrease of 37.18%. Furthermore, results also indicate a significant superiority of M<sub>2</sub> treatment (38.21 g plant<sup>-1</sup>) over the means of M<sub>1</sub>, G<sub>2</sub> and control treatment with percentages of 13.24, 15.12 and 39.19%, respectively, then following by G<sub>2</sub> treatment (36.30 g plant<sup>-1</sup>).

**Table (1):** Effect of shading and some plant extracts on the leaves' dry weight (g plant<sup>-1</sup>).

Plant extracts	Shading		Mean
	S <sub>1</sub>	S <sub>2</sub>	
M <sub>0</sub> G <sub>0</sub>	32.67	22.23	27.45
M <sub>1</sub> G <sub>0</sub>	42.17	25.31	33.74
M <sub>2</sub> G <sub>0</sub>	47.47	28.94	38.21
M <sub>0</sub> G <sub>1</sub>	40.61	25.77	33.19
M <sub>0</sub> G <sub>2</sub>	44.54	28.05	36.30
LSD <sub>0.05</sub>	3.08**		2.18*
Mean	41.49	26.06	
<b>LSD<sub>0.05</sub></b>	<b>2.57**</b>		

The results of the Table 1 showed a significant interaction, as the highest combination was recorded between S<sub>1</sub> and M<sub>2</sub>, amounted to 47.47 g plant<sup>-1</sup>, followed by the S<sub>1</sub> and G<sub>2</sub> which amounted to 44.54 g plant<sup>-1</sup>, with non-significant difference between them, and the lowest leaf dry weight was recorded between S<sub>2</sub> and the control treatment (22.23 g plant<sup>-1</sup>).

Stevia plant stress resulting from low light affects the biomass in the plant due to a decrease in the plant's photosynthetic capacity and changes in the function of stomatal cells on the leaf surface, affecting gas exchange and carbon fixation. Therefore, high levels of shading should be avoided, and cultivation with a shade level below 50% is recommended to achieve high dry weight (Kumar *et al.*, 2012). Under intense light radiation, which affects gene expression, there is an improvement in photosynthetic activity and an increase in carbohydrate production and accumulation in the leaves, leading to an overall biomass increase in the plant (Bote *et al.*, 2018). Moringa leaf extract is rich in plant hormones such as indole-3-acetic acid (IAA), gibberellins, and zeatin cytokinin, which contribute to improving plant growth and the accumulation of carbohydrates and salts (Al-Taweel & Al-Anbari, 2019), which leads to increased vegetative and root growth in Stevia, thereby enhancing water and nutrient uptake



from the soil, which is then transferred to the leaves to improve crop growth, ultimately resulting in increased fresh and dry weight of the plant (Casanova *et al.*, 2009). Furthermore, the presence of sugars in the extract of Licorice roots increases the osmotic pressure of cells, thereby enhancing the absorption of water and other nutrients, positively impacting overall yield. Licorice root extract is rich in potassium, which is known to regulate and stimulate physiological processes in plants, including its effect on photosynthesis and the transfer of its products, which stimulate ATP synthesis needed by the plant for various physiological activities, to storage sites in the plant.

Additionally, potassium plays a role in the accumulation of sugars, amino acids, protein formation, and carbohydrate accumulation (Musa *et al.*, 2002). required for vegetative growth and increasing economically important leaf yield. Additionally, the availability of nutrients is crucial (Yoneda *et al.*, 2017). Analyses conducted on Moringa leaf extract revealed the presence of macro and micro essential elements such as Ca, Mg, K, P, Fe, Mn, Cu, and Zn, which contribute to the development and improvement of vegetative growth in Stevia plants (Jain *et al.*, 2020). Foliar application of Licorice root extract, rich in active and nutritious components and containing gibberellin, played a significant role in stimulating cellular activities, resulting in cell enlargement and division, leading to increased leaf growth and branching in the plant (Babilie *et al.*, 2015).

#### Leaves dry weight yield (ton ha<sup>-1</sup>)

The results indicate a significant difference between the studied factors and significant interaction in the dry weight yield of leaves per hectare (Table 2). The results indicate that the mean of S<sub>2</sub> by 37.20% compared to S<sub>1</sub>. The results also demonstrate a significant superiority of M<sub>2</sub> treatment, which reached 6.33ton ha<sup>-1</sup>, over the M<sub>1</sub>, G<sub>1</sub> and M<sub>0</sub>G<sub>0</sub> treatments by 13.23, 17.43 and 39.12% respectively, followed by G<sub>2</sub> treatment, which reached 6.13 ton ha<sup>-1</sup>.

**Table (2):** Effect of shading and some plant extracts on the leaves dry weight yield (ton ha<sup>-1</sup>).

Plant extracts	Shading		Mean
	S <sub>1</sub>	S <sub>2</sub>	
M <sub>0</sub> G <sub>0</sub>	5.42	3.68	4.55
M <sub>1</sub> G <sub>0</sub>	6.99	4.20	5.59
M <sub>2</sub> G <sub>0</sub>	7.87	4.80	6.33
M <sub>0</sub> G <sub>1</sub>	6.51	4.27	5.39
M <sub>0</sub> G <sub>2</sub>	7.61	4.65	6.13
LSD <sub>0.05</sub>	0.64*		0.45**
Mean	6.88	4.32	
LSD <sub>0.05</sub>	0.24**		

The results in Table 2 indicate that there was a significant interaction. The highest combination of interaction between S<sub>1</sub> and M<sub>2</sub> was recorded at 7.87ton ha<sup>-1</sup>, followed by the combination of interaction between S<sub>1</sub> and G<sub>2</sub>, amounted to 7.61ton ha<sup>-1</sup> with non-significant difference between them, whereas the lowest interaction between S<sub>2</sub> and control treatment (M<sub>0</sub>G<sub>0</sub>) was 3.68 ton ha<sup>-1</sup>.

The results in Table 2 illustrates that obtaining dry biomass under sunlight is significantly higher for the dry leaf yield compared to shaded plants. Increased shading levels





resulted in delayed growth and increased time required to reach various stages of plant development in Stevia. It is recommended to reduce shading levels to achieve a high dry weight yield per unit area (Kumar *et al.*, 2012). Additionally, the extract of Moringa leaves, rich in cytokinins, plays an effective role in maintaining leaf area, leaf count per plant, and maximizing photosynthesis. This leads to increased plant size in the field and the preservation of leaves from senescence (Jain *et al.*, 2020). Babilie *et al.* (2015) indicated that the sugar compounds, organic compounds, and major nutrients such as potassium, phosphorus, magnesium, and trace elements present in Liquorice root extract, which contains the gibberellin precursor hormone, contributed to increasing the accumulation of dry matter in the plant by maximizing carbohydrate accumulation. Consequently, the results were consistent and aligned with an increase in leaf count, length, and area per plant and an increase in fresh weight of the total vegetative mass, ultimately resulting in a higher percentage of dry weight in cultivated lands.

### Total Antioxidant Activity (mg L<sup>-1</sup>) (Ascorbic acid equivalent)

The results in Table 3 demonstrate a significant difference between the factors and significant interaction between the studied factors in the total antioxidant activity. The results showed that the S<sub>2</sub> significantly surpassed S<sub>1</sub> by 71.95%. The results also indicate that the means of M<sub>2</sub>, G<sub>1</sub>, M<sub>1</sub> and control treatment decreased considerably compared to the mean of G<sub>2</sub> by 66.95, 54.81, 22.87 and 16.95%, respectively.

**Table (3):** Effect of shading and some plant extracts on the total antioxidant activity (mg L<sup>-1</sup>).

Plant extracts	Shading		Mean
	S <sub>1</sub>	S <sub>2</sub>	
M <sub>0</sub> G <sub>0</sub>	9319.00	12029.00	10674.00
M <sub>1</sub> G <sub>0</sub>	7853.00	11971.00	9912.00
M <sub>2</sub> G <sub>0</sub>	2755.00	5739.00	4247.00
M <sub>0</sub> G <sub>1</sub>	3876.00	7740.00	5808.00
M <sub>0</sub> G <sub>2</sub>	8183.00	17522.00	12852.50
LSD <sub>0.05</sub>	311.90**		220.50**
Mean	6397.20	11000.20	
LSD <sub>0.05</sub>	123.90**		

The results in Table 3 indicated a significant interaction between the studied factors. The highest combination recorded between S<sub>2</sub> and G<sub>2</sub>, amounted to 17522.00 mg L<sup>-1</sup>, and the lowest combination was between S<sub>1</sub> and M<sub>2</sub> at 2755.00 mg L<sup>-1</sup>.

Decreases light optimum conditions by shading may increase the total antioxidant activity in Stevia during the studied crop growth period. This increase is likely due to reduced photosynthesis efficiency under shading. Shading can potentially enhance the activities of antioxidant compounds in Stevia against less light stress, as antioxidants are produced as secondary metabolites in cells not exposed to environmental stress and through respiratory processes in the leaf (Rady *et al.*, 2019).

Moreover, the biostimulants, such as Liquorice root extract, impact the contents of phenols, flavonoids, and pyruvic acid, as well as the overall capacity of antioxidants and their increased production in the plant. This is attributed to the presence of various chemical



elements in the Liquorice root extract, such as Mg<sub>2</sub> and Fe<sub>2</sub>, along with other elements and stimulants that arise from natural metabolic pathways, stimulating plant growth, development and stress tolerance when applied at specific concentrations (Rouphael & Colla, 2018).

#### Total phenols (mg L<sup>-1</sup>) (Gallic acid equivalent)

The results in Table 4 demonstrate a significant difference between the studied factors in the total phenolic concentration in the leaves and the presence of a significant interaction between the studied factors in this trait. The results reveal a significant superiority of the S<sub>2</sub> mean over S<sub>1</sub>, with a percentage difference of 14.49%. Furthermore, all means of M<sub>2</sub>, G<sub>1</sub>, G<sub>2</sub> and M<sub>0</sub>G<sub>0</sub> show a decrease compared to the mean of M<sub>1</sub>, with percentages of 85.54, 70.98, 24.47 and 30.25, respectively.

The results in Table and Figure 4 indicate a significant interaction. The highest combination recorded between S<sub>2</sub> and M<sub>1</sub>, amounted to 20288 mg L<sup>-1</sup>, and the lowest interaction combination was between S<sub>2</sub> and M<sub>2</sub>, which amounted to 1764 mg L<sup>-1</sup>.

**Table (4):** Effect of shading and some plant extracts on the total phenol (mg L<sup>-1</sup>).

Plant extracts	Shading		Mean
	S <sub>1</sub>	S <sub>2</sub>	
M <sub>0</sub> G <sub>0</sub>	12021.00	10487.00	11254.00
M <sub>1</sub> G <sub>0</sub>	11982.00	20288.00	16135.00
M <sub>2</sub> G <sub>0</sub>	2902.00	1764.00	2333.00
M <sub>0</sub> G <sub>1</sub>	4683.00	4681.00	4682.00
M <sub>0</sub> G <sub>2</sub>	11854.00	12517.00	12185.50
LSD <sub>0.05</sub>	563.80**		398.70**
Mean	8688.40	9947.40	
LSD <sub>0.05</sub>	321.70**		

Sugars, aromatic amines, sulfur dioxide, ascorbic acid, organic acids, and others can react to create high phenolic concentrations by increasing photosynthesis while reducing environmental stress through shading (Prior *et al.*, 2005). The potential of moringa extract, which is an important bio-stimulant for enhancing plant growth and biological activities, lies in its ability to increase the concentration of hormonal contents and mineral nutrients, as well as its role in providing zeatin, ascorbic acid, calcium, and potassium, all of which contribute to increasing phenolic concentrations and numerous antioxidants. Foliar spraying enhances the plant's ability to increase the uptake of essential elements, thereby increasing the nutritional value of Stevia plant leaves. The antioxidant activity in Moringa is due to its high content of phenolic compounds, which are essential for the growth and defense of Stevia plants against diseases (Al Taweel & Al-Anbari, 2019). Bio-stimulants primarily enhance crop productivity by improving root system structure, enhancing plant water and nutrient uptake, and optimizing photosynthesis by improving chlorophyll content and its role in maximizing growth and the antioxidant defense system while reducing oxidative stress in plants (Shah *et al.*, 2019).

#### Leaves content of chlorophyll a (mg 100 g<sup>-1</sup> fresh weight)



The results in Table 5 reveal that there were no significant differences between shading treatments ( $S_1$  and  $S_2$ ) in the leaves content of chlorophyll a, while there was a significant difference between plant extract treatments in this trait, as  $M_1$  was significantly superior and gave the highest mean at 189.45 mg 100 g<sup>-1</sup> fresh weigh, followed by  $G_1$  and  $M_2$  which gave 188.55 and 180.55 mg 100 g<sup>-1</sup> fresh weigh respectively, compared to  $G_2$  and control treatment. The results in Table 5 and Figure 5 indicate that there was a significant interaction between studied factors in this trait, as the highest interaction recorded between  $S_1$  and  $M_2$ , reached 212.00 mg 100 g<sup>-1</sup> fresh weight with a non-significant difference with the interaction between  $S_2$  and  $M_1$ , which reached 205.30 mg 100 g<sup>-1</sup> fresh weight. In contrast, the lowest value observed between  $S_1$  and  $G_2$ , reached 134.90 mg 100 g<sup>-1</sup> fresh weight.

**Table (5):** Effect of shading and some plant extracts on the leaves content of chlorophyll (mg 100 g<sup>-1</sup> fresh weight).

Plant extracts	Shading		Mean
	$S_1$	$S_2$	
$M_0G_0$	171.50	147.80	159.65
$M_1G_0$	173.60	205.30	189.45
$M_2G_0$	212.00	149.10	180.55
$M_0G_1$	189.30	187.80	188.55
$M_0G_2$	134.90	191.10	163.00
LSD <sub>0.05</sub>	20.65**		14.60**
Mean	176.26	176.22	
LSD <sub>0.05</sub>	N.S		

Foliar spraying with Moringa leaf extract enhances chlorophyll content and the rate of photosynthesis (Hoque *et al.*, 2020), attributed to the role of Moringa extract in increasing gas exchange and stomatal conductance, along with its content of growth hormones such as cytokinins, gibberellins, and auxins, which promote plant growth and increase its green surface area, thereby enhancing photosynthesis (Abdalla, 2013). Furthermore, the Liquorice root extract containing gibberellin precursors plays a positive role in the plant's bio-building and activates chlorophyll pigmentation (Al-Abdali, 2002).

#### fresh leaves content of chlorophyll b (mg 100 g<sup>-1</sup> fresh weight)

The results indicate a significant difference between shading treatments and a significant interaction between the two studied factors in the leaves' chlorophyll b content (Table 6). The mean of  $S_2$  was significantly superior by 8.70% compared to  $S_1$ . There were no significant differences between the means of the plant extract treatments.

The results in Table 6 and Figure 6 show that there was a significant interaction between the two studied factors in this trait, as the highest combination recorded between  $S_2$  and  $G_2$  (91.10 mg 100 g<sup>-1</sup> fresh weight) with non-significant difference with the combinations of  $S_2G_1$  and  $S_1M_2$  which recorded 81.30 and 81.00 mg 100 g<sup>-1</sup> fresh weight respectively, while the lowest value was observed between  $S_1$  and  $G_2$  which was 52.70 mg 100 g<sup>-1</sup> fresh weight.



**Table (6):** Effect of shading and some plant extracts on the leaves content of chlorophyll b (mg 100 g<sup>-1</sup> fresh weight).

Plant extracts	Shading		Mean
	S <sub>1</sub>	S <sub>2</sub>	
M <sub>0</sub> G <sub>0</sub>	70.00	64.50	67.25
M <sub>1</sub> G <sub>0</sub>	70.70	76.30	73.50
M <sub>2</sub> G <sub>0</sub>	81.00	67.60	74.30
M <sub>0</sub> G <sub>1</sub>	75.90	81.30	78.60
M <sub>0</sub> G <sub>2</sub>	52.70	91.10	71.90
LSD <sub>0.05</sub>	10.95**		N.S
Mean	70.06	76.16	
LSD <sub>0.05</sub>	2.73*		

Increasing shading reduces photosynthesis and plant growth and increases crop plants' stomatal and mesophyll resistance to gas exchange. Additionally, shading reduces leaf thickness due to the formation of a thin layer of barriers that allows the plant to suffice with a minimal amount of light for photosynthesis and gas exchange (Nygren & Killomaki, 1993). However, under shading conditions and due to low plant density, the reduced spacing between plants may diminish the shading effect and decrease competition among plants in the Stevia cultivation ecosystem, thereby enhancing photosynthetic efficiency and delaying leaf senescence (Ramesh *et al.*, 2007).

#### Leaves content of total chlorophyll (mg 100 g<sup>-1</sup> fresh weight)

The results indicate there were non-significant differences between the studied factors. In contrast, the interaction between them significantly affected the leaves content of total chlorophyll, as the results in Table 7 and Figure 7 show that the S<sub>1</sub>M<sub>1</sub> combination recorded a highest value (292.90 mg 100 g<sup>-1</sup> fresh weight). In contrast, the S<sub>1</sub>G<sub>2</sub> combination recorded the lowest value (187.50 mg 100 g<sup>-1</sup> fresh weight).

**Table (7):** Effect of shading and some plant extracts on the leaves content of total chlorophyll (mg 100 g<sup>-1</sup> fresh weight).

Plant extracts	Shading		Mean
	S <sub>1</sub>	S <sub>2</sub>	
M <sub>0</sub> G <sub>0</sub>	241.40	205.60	223.50
M <sub>1</sub> G <sub>0</sub>	244.20	281.60	262.90
M <sub>2</sub> G <sub>0</sub>	292.90	216.70	254.80
M <sub>0</sub> G <sub>1</sub>	265.20	269.10	267.15
M <sub>0</sub> G <sub>2</sub>	187.50	282.20	234.85
LSD <sub>0.05</sub>	54.12**		NS
Mean	246.24	251.04	
LSD <sub>0.05</sub>	N.S		

#### CONCLUSION

Based on the results concluded that Stevia plants grown in the open field (without shading) produced the highest leaves dry weight, leaves content of chlorophyll b, and leaves



yield per unit area, while the Stevia plants grown in the 50% shading had the highest values of total antioxidant activity, total phenols and leaves content of chlorophyll a. Also, spraying moringa extracts at a concentration of 15% made the highest leaves dry weight, leaves content of chlorophyll b, and leaves yield per unit area while spraying it at 10% produced the highest leaves content of chlorophyll a. However, the spraying of Liquorice root extract at a concentration of 6% had the highest values of total antioxidant activity and total phenols. Therefore, it is recommended to plant Stevia plants in the 50% shading and spraying of Liquorice root extract at the concentration of 6% to increase the total antioxidant activity and phenols.

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