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Effect of biofertilizers in improving production of hot pepper (*Capsicum annuum* L.), and tolerating drought stress

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ABSTRACT

Chili peppers (*Capsicum annuum* L.) are one of the most widely planted vegetables all over the world since it has several health advantages and add a delicious flavor to a range of recipes. Drought stress is accounted as the most abiotic environmental condition that can negatively impact the quality and production of many crops. In this context, a factorial randomized design study was conducted in open field pots to determine how irrigation deficit affects the plant's agronomic and physiological characteristics. *Capsicum* spp. was subjected to three drought stress conditions (once every day, once every two days, and once every three days representing light or low, moderate, and severe drought stress). At the same time, biostimulants Alga600, moringa leaf extract (MLE), and homemade fertilizer (H-made) were foliar sprayed to determine whether they might minimize abiotic stress or increase plant susceptibility to drought. The results demonstrate that severe drought stress resulted in a considerable drop in fruit quality and production, followed by moderate and light drought. Whereas, minimum chlorophyll and carotenoids are influenced by deficient irrigation. On the other hand, biostimulants could mitigate the detrimental impact of drought stress on yield, chlorophyll concentration, and other physical characteristics such as leaf area. Among the biostimulants, Alga600 had the greatest impact on increasing chili pepper stress tolerance, followed by 6% MLE and H-made biostimulants when compared to their controls (plants treated with deionized water only). However, the combination of biostimulants with drought stress was more obvious in drought roses. The interaction was less influenced by the drought, which intensified the physical and economic aspects. While some biostimulants, such as MLE and Alga600, may have a greater impact on plant production and pigment content as drought stress increases. The correlation coefficient also indicates that the measured parameters had both positive and negative correlations to each other. Physical parameters like leaf area, number of fruits, fruit diameter, and length have a significant positive correlation to the total yield while chlorophyll content can have a positive and negative correlation to the physical parameters.

1.Introduction

Chili peppers have a significant commercial value in the Iraqi markets. It is a highly demandable vegetable among the people, which is consumed fresh, dried, and fermented in pickles. The plant grows throughout the country, but water scarcity and climate change have impeded the plant's ability to thrive in some parts of the country. Compared to other horticultural plants, vegetables are short-season crops that require a lot of water and a continuous supply of irrigation water to stay sustained (Roux *et al.*, 2016). Furthermore, in Iraq, there is a limited amount of area accessible for growing vegetables and fruits, estimated at less than 0.5 million hectares out of 8 million total cultivated land. So that Iraqi farmers grow fewer vegetables instead they cultivate plants that need less water or depend on seasonal rainfall called (Dim irrigation) such as wheat, barley, and some kinds of summer vegetables like Armenian cucumber, Cucumis melo, Okra, etc as well as winter vegetables as lettuce, cabbage, cauliflower which they rely on autumn rainfall (Jaradat, 2003).
Chili peppers are accounted for a valuable source of vitamins (A, C, and E), and numerous antioxidant agents, and are utilized as a natural taste and color in foods. They also have numerous medical benefits, including anti-inflammatory, anti-allergic, anti-allergenic, anti-carcinogenesis and anticoagulant properties. It was discovered that eating ripe chili peppers reduces the incidence of cancer and can help prevent blood clots (Chakrabarty *et al.*, 2017, Idrees *et al.*, 2020, Nazeer *et al.*, 2023).
Drought currently plays a vital part in agriculture crop loss, because it affects surface and subsurface water, as well as water quality (Ahmadalipour and Moradkhani, 2017, Van Loon *et al.*, 2014). Also, drought has impacted the way farmers grow in Iraq. Iraq ranks sixth among the countries most impacted by climate change, according to statistics from the United Nations (Nation, 2022). Iraq is increasingly reliant on surface water from the two main rivers (Tigress and Euphrates), which is currently reducing the loss of 8-12 million cubic meters of surface water annually (UNDP, 2023), as a result of climate change (limited yearly precipitation and the construction of dams in the rivers' source areas) led to limitation of water flow into these two main rivers from upstream. This problem jeopardizes the country's agricultural sustainability. Many agricultural industries have been forced to adopt commodities that use less water, and some fishing enterprises have been dismantled due to a scarcity of drinking water and an overall decrease in the use of surface and groundwater. Climate change is anticipated to have a significant impact in the Middle East, leading to more intense and frequent droughts (Hameed *et al.*, 2018). As a result, scientists are increasingly focused on finding a way to reduce the impact of drought on crop yield.
Peppers are more susceptible to drought stress during the vegetative growth stage, as compared to blooming and fruit set (Phimchan *et al.*, 2012). Researchers have demonstrated that drought stress reduces physiological properties in chili peppers; however, it increases the pungency and more capsaicin in the fruit (Ichwan *et al.*, 2017, Kopta *et al.*, 2020, Okunlola *et al.*, 2017).
Chili peppers grow and produce well in the day temperature between 18 and 27 ℃ and night temperature between 15 and 18 ℃ (Campiglia *et al.*, 2011), however, in Iraq, the temperature reaches above 45℃ in the pepper growing season. This requires extra watering in places with limited water supplies, the applied amount of water can only be reduced by about 20% without causing substantial yield loss (Barbero *et al.* 2016). Drought stress has largely affected summer vegetables in Iraq, due to a shortage of fresh water to irrigate the vegetables regularly.
Many research studies have demonstrated that adding biostimulants, mycorrhizal fungi and some type of fertilizers have a positive influence on quality improvement and reduction of drought stress on plants as in sweet yellow pepper (*Capsicum annum* L.) (Ichwan *et al.*, 2021, Parađiković *et al.*, 2011), tomatoes (*Lycopersicon esculentum* L.) (Goñi *et al.*, 2018), eggplant (*Solanum melongena* L.) (Semida *et al.*, 2021), Okra (*Abelmoschus esculentus* L.) (Ali *et al.*, 2024), lettuce (*Lactuca sativa* L.) (Yaseen and Takacs-Hajos, 2022a, Yaseen and Takácsné-Hájos, 2021), Onions (*Allium cepa* L.) (Yaseen & Takácsné Hájos 2020), however, very little research has been presented on peppers,

whereas the productivity of chilli peppers (*Capsicum annuum*) is threatened by drought stress (Bulle *et al.*, 2024). Protein and osmolyte accumulation in plants is responsible for adaptation and adjustment to drought conditions (Gagné-Bourque *et al.*, 2016). Other researchers have shown the importance of some macronutrients as potassium in regulating cell division and cell elongation in plants.

Today, Iraq is ranked as the fifth most climate-vulnerable country in the world, and it faces severe difficulties due to rising temperatures, drought or less seasonal precipitation, increased salinity, and frequent dust storms (International Organization for Migration 2022). Many studies have been conducted in the country's central and southern regions to improve plant responses to diverse abiotic stresses. However, relatively little study on drought stress on vegetable crops has been conducted in the northern part (Kurdistan region) of the country. This could be because the drought has had less of an impact in this area of the country; nonetheless, scientists are warning that if action is not taken quickly, the entire country could face a significant threat in the coming decades. Furthermore, to determine how farmers or low-income folks may minimize the problem of drought or water scarcity in their field without having to spend a lot of money, we wanted to evaluate a biostimulants made at home and compare it to biostimulants that are readily accessible locally. This is because only biostimulants that are marketed for sale have been shown to reduce or raise plant susceptibility to water shortage in this country. As a result, the goal of this research is to develop a technique to possibly mitigate the effects of climate change, particularly drought, on the production of hot peppers. As a result, we are focusing on cultivating chili pepper at various drought stress levels to explore if biostimulants help alleviate drought stress.

2. Materials and Methods

2.1 Experimental design

The experiment was conducted in South East Erbil, Northern Iraq (Figure 1). The experimental design consisted of a factorial complete randomized design CRD. Seedlings of the Bursa seed company's ILICA 256 variety were transplanted into pots (30 cm width, 35 cm length, and 25 cm height) at the end of April 2023, some metrological data of the air temperature and humidity during the growing season are shown in table 1. Each container held one plant. Irrigation with 250-300 mL of water was scheduled at three distinct treatment levels (once a day (light or mild drought), once every two days (moderate drought), and once every three days (severe drought), based on field capacity.

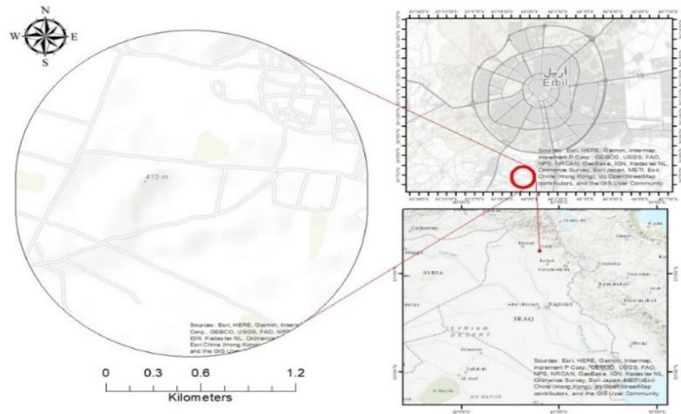

Figure 1. Map of the study location

The pots were filled evenly with 1:2:1 perlite, loam, and compost and placed in an open field in the climate condition. Pots were divided into three replications, each of which required six pots. One more potted plant was put around each replication as a guard plant, but no data was collected. The quantity and watering schedule are displayed as follows:

- *Mild drought (tap water) – irrigate once a day 250-300 mL plant^{-1}*
- *Moderate drought (tap water) – irrigate every 2 days 250-300 mL plant^{-1}*
- *Severe drought (tap water) – irrigate every 3 days 250-300 mL plant^{-1}*

Table 1: Air metrological data (minimum, maximum, and average temperature and humidity) of the study location during the growing season of the peppers

Months	Temperature ℃			Humidity %		
	Min	Max	average	Min	Max	average
April	4	32	19	14	95	54
May	12	53	26	6	96	36
June	21	42	31	7	94	27
July	25	47	36	5	47	19
August	25	48	37	4	48	17
September	19	44	31	6	50	25
October	15	34	24	12	86	44
November	4	30	17	25	98	68
December	1	22	12	27	100	83

2.2 Plant materials and foliar treatments

Plants were foliar treated with (Alga600 6%, Moringa leaf extract (MLE) 6%, and Home-made 10% biostimulants) starting two weeks after transplanting and continuing every two weeks for four times with the amount of (15-30 mL plant^{-1}) as follows:

- *Control Ø (foliar treated with deionized water only)*
- *Alga600 (0.04g pot^{-1}) 2 ml L^{-1} or 2 g L^{-1}*
- *MLE 6% 20 mL plant^{-1}*
- *Home-made biofertilizer 10 %, 20 mL plant^{-1}*

2.3. Biostimulant preparation

2.3.1 Alga600

Dissolve 30g of Alga600 in 100 L deionized water then plant foliar sprayed with 20 mL plant^{-1} or till leached from the leaves after adding a few drops of tween 20. The Alga composition is shown in the Table 2:

Table 2. The composition of Alga600 foliar sprayed onto hot peppers

pH	5.2
EC (dS m^{-1})	4.8
Organic matter	35%-40%
Alginic Acid	12%-15%
Potash (K_2O)	17%

2.3.2 Moringa leaf extract (MLE)

After 40 days, new moringa leaves were harvested and dried in a shaded area. According to the procedure by Makkar and Becker, (1996), 20g of leaf powder was measured, and 250 mL of 98% ethanol was added every three hours until it reached 675 mL of liquid. The mixture was then left for 24 hours. Every two weeks four times, 6% MLE was foliar sprayed into plants based on this biostimulant. We refrigerated the remaining prepared liquid until we needed it. Previous studies have shown the richness of the *Moringa oleifera* Lam. leaf extract as shown in Table 3:

Table 3. Mineral element content in 100 g moringa leaf extract (MLE).

Mineral content	Value (mg)
Phosphorus (P)	100
Potassium (K)	350
Sulphur (S)	267
Calcium (Ca)	326
Magnesium (Mg)	86.8
Sodium (Na)	11.4
Iron (Fe)	2.24
Cupper (Cu)	0.319
Zinc (Zn)	0.477
Boron (B)	0.581

Yaseen and Takacs-Hajos (2022b)

2.3.3 Home-made biostimulant preparation

The 250g of banana peel and 250g of eggplant were mashed in a kitchen blender, which served as a source of potassium, calcium, iron, and many macro and microelements (Agency, 2002). A very fine powder was created from blending gathered eggshells that had been dried out in the sun for a week. To remove the minerals from the shell, 1 L of apple vinegar (150g Eggshell powder) was added to the powder. The extract was placed in a shaded area to dry out after consistent shaking for approximately 15 days. The smashed eggplant and banana were then mixed with the prepared eggshell powder. To add microorganisms, 60 g of yeast was added to the prepared materials. After mixing 1 kg of soil in 5 L of water, the mixture was kept in a shaded area for fermentation for two weeks while being stirred twice a day. The prepared extract was diluted to 10% and applied as a foliar spray directly to the chili peppers every two weeks for four treatments after being sieved through a very fine mesh fabric material. Some of the mineral contents of the Home-made biofertilizer were measured which is shown in the below table:

Table 4. Mineral content in the Home-made biofertilizer in 100mL of the liquid

Mineral content	*Value (mg)*
pH	*6.2*
EC (dS m^{-1})	*5.04*
Nitrogen (N)	*146*
Phosphorus (P)	*4.2*
Potassium (K)	*1.8*
Calcium (Ca)	*0.326*
Iron (Fe)	*35.8*
Boron (B)	*1.25*
Sulphur (S)	*0.063*
Organic matter	*224*

2.4. Sample collection and measurements

Samples were collected at the intermediate ripening stage at a full size where the fruit contains the maximum amount of flavonoids, antioxidants, and polyphenols based on the recommended ripening stage by Shaha *et al.* (2013) for fresh produce of hot peppers. Morphological parameters (leaf area, number of fruits, fruit weight per plant, length, and diameter of fruit, total yield per donum) were measured in this regard. In addition, several laboratory measures (chlorophyll a, b, and total chlorophyll, carotenoids, and chlorophyll ratio) were also taken. The chlorophyll concentration was measured using a spectrophotometer based on the destructive method by Ali *et al.* (2021). To conduct the 80% acetone solvent procedure, 0.25g of leaf sample was ground in 2ml of 80% acetone with 0.1% or 0.1 $CaCO_3$ to prevent chlorophyllase activity. The sample was then filtered through filter papers and the final volume was 25ml. The absorbency of the sample (A_{663}, A_{646}, and A_{470}) was calculated using the spectrophotometer after being in a centrifuge for15 minutes.

The calculation was as follows:

Chl a = 12.25 (A_{663}) – 2.79 (A_{646}) …………… (1)

Chl b = 21.5 (A_{646}) – 5.1 (A_{663}) …………. ….. (2)

$$\text{Total Chlorophyll} = \frac{5.24x(A_{663}) - 22.24x\,(A_{646})}{FW}$$ ………. (3)

$$\text{Carotenoids} = \frac{1000x\,(A_{470}) - 1.82\,Chl\,a - 85.02\,Chl\,b}{198}$$ ……. (4)

Leaf area index (LAI)

The leaf area was measured using a free software called ImageJ. The leaf was photographed with 4-cm^2 red calibration area using a camera phone (iPhone 13 ProMax, Apple). The method was followed by Schneider *et al.* (2012). To do this, the leaves from the plant's 5th internode were taken, which was around 50 cm tall, using 4 leaves from the plant's back, front, north, and south directions. The average leaf area index (LAI) was derived using the collected data. Measured software usage is shown in Figure 2.

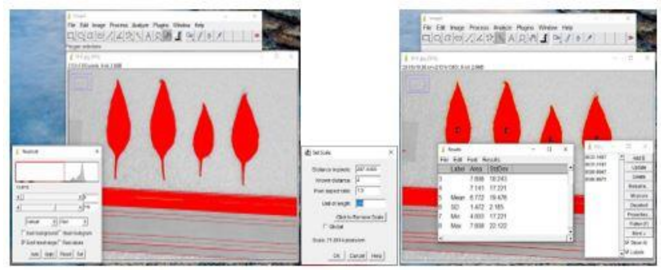

Figure 2. Leaf area screening by ImageJ software

2.5. Statistical Analysis

Data was subjected to a two-way analysis of variance (ANOVA) using SPSS version 25 (SPSS Inc., Chicago, IL, USA). Differences among means were calculated using the Duncan multiple range test at $\rho \le 0.05$. Some correlation and regression analyses were also carried out to see the correlation among the factors using the correlation coefficient (or Pearson correlation coefficient).

3.0. Results

3.1. Chlorophyll content in relation to drought stress

Green plants have larger levels of chlorophyll a and b than carotenoids and other pigments, particularly during the growth season. Green plant leaves have three times more chlorophyll a than chlorophyll b. Our result shows a significant reduction of chlorophyll a, b, and total chlorophyll content at the wavelength of 663 and 645 for chlorophyll a and b respectively. While, the ratio of chlorophyll a/b was significantly increased with the increase of drought stress from low drought to severe drought stress (Figure 3a, b, and c).

Drought stress causes a reduction in chlorophyll a and b, which makes plants less able to absorb light and, as a result, less able to provide food for other portions of the plant to produce fruits (Li *et al.*, 2008).

Carotenoids, on the contrary, reduction of total chlorophyll (Chl a+ Chl b) led to a significant increase of carotenoids at (*P*≤0.05) in pepper leaves under severe drought stress (figure 3e). Plants under moderate water stress also had greater carotenoid content but no significant levels were detected. This result agrees with the result by Iqbal *et al.* (2023) where they found significantly higher carotenoids in sweet peppers under 30% field capacity drought than 50% and the control. Zhang *et al.* (2021) indicate that increases in carotenoids especially *β*-carotene under drought stress are much related to the gene expression of DcPSY2 and DcLCYB in plants.

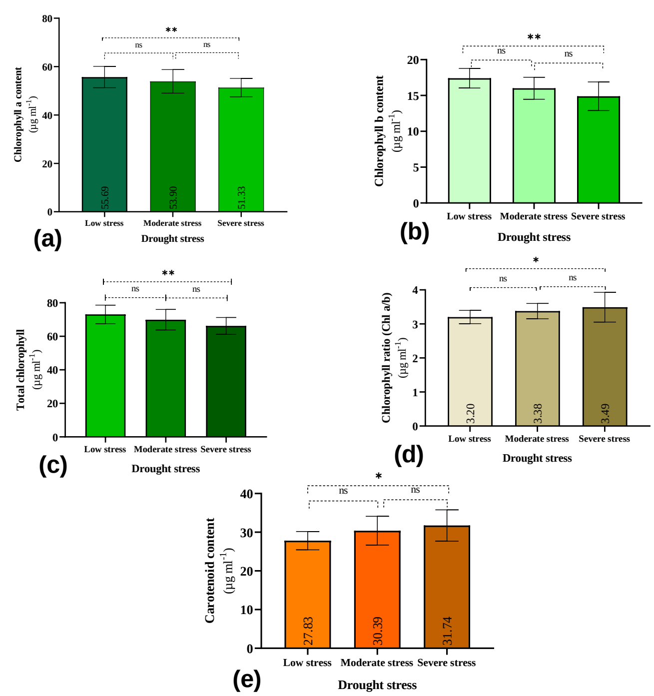

Figure 3. Chlorophyll a, b and carotenoids content in the pepper leaves under drought stress where (a) is the Chlorophyll a under low, moderate, and severe drought stress, (b) Chlorophyll a under same drought stress, (c) total chlorophyll (a+b) which has been calculated based on the standard formula, (d) Chlorophyll ration Chla/ Chlb and Carotenoids in (e) Figure.

3.2. Physical parameters related to drought stress

The results in Figure 4 reveal that drought stress caused a considerable decline in growth parameters. The physical characteristics decreased more as the drought regime increased. Figures 3a and c demonstrate a substantial reduction (*P≤0.05*) and (*P≤0.01*) of fruit diameter and fruit length in plants under mild, moderate, and severe drought stress. Figure 3b shows similar results for the number of fruits per plant; the only difference is that no significant differences between moderate and severe drought were identified. A key indicator of how plants adapt to changes in the environment and track their growth over time is leaf area. Leaf area dramatically decreased as water scarcity increased, in line with earlier findings. This is because drought stress prevents leaf area from expanding (Widuri *et al.*, 2017). Many other research studies indicate that water deficient or drought stress reduces leaf area significantly as in cluster beans *(Cyamopsis tetragonoloba L.* Taub*)* (Shubhra *et al.*, 2003), and cucumber (Li *et al.*, 2008).

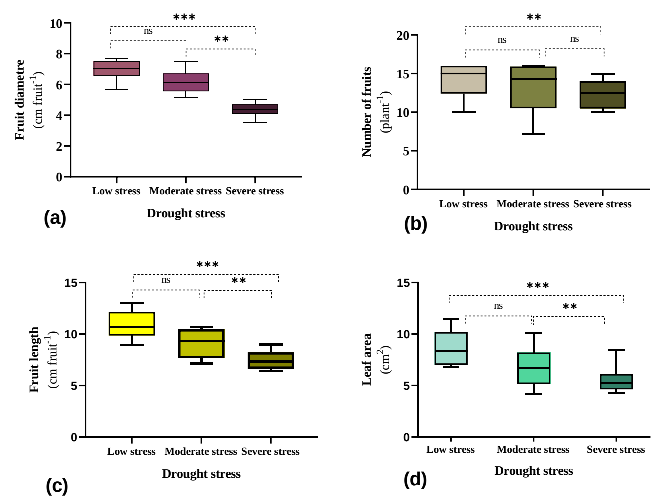

Figure 4. Effect of different drought stress levels on fruit diameter (a), number of fruits per pants (b), fruit length (c), and leaf area (d) in chili peppers after eight weeks of drought stress (during the vegetative growing season). The number of samples per point, n=3. Different letters in each chart indicate significant differences between the treatments using the Duncan multiple range test ((*P≤0.05*) for ***and (*P≤0.01*) for **).

3.3. Fruit weight and Total yield related to drought stress

The weight of fruits per plant and total production per donum in chili peppers reduced significantly as drought stress aggravated. When the irrigation shortage increased from once a day to every three days, the fruit weight per plant decreased. The average fruit weight reflects a higher overall yield since it is directly proportional to the product's ultimate yield. Figure 3a shows a significant drop in fruit weight between watering the plant once a day and once every three days at (*P≤0.05*), with differences of -6% and -13% for moderate and severe drought stress compared to the control (once per day). Also, the study found that increasing drought stress from once every two days to once every three days reduced the total yield per donum by -5% and -15%, respectively. Figure 3 (b) reveals a substantial reduction in pepper weight at (*P≤0.01*) between low and severe irrigation stress, but moderate stress showed no significant difference with neither low nor severe stress, yet the weight loss was -5%.

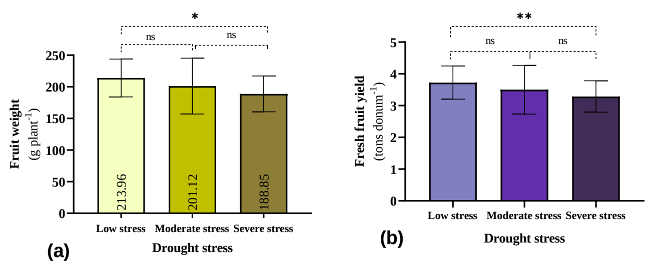

Figure 5. Low, moderate, and severe drought stress influence fruit weight per plant (a) and total fresh yield tons per donum in chili peppers after facing the plant to irrigate water in three different drought stress levels (once a day, once per two days, and once per three days).

3.4. Impact of biostimulants on some physical parameters and yield of chili peppers

Biostimulants had less of an impact on the physical characteristics and production of chili peppers than on untreated plants. Table 5 shows that homemade (H-made) biostimulant had much lower readings than Alga600 and moringa leaf extract (MLE), which had the largest fruit diameter and fruit length among the assessed physical characteristics. This is because Alga600 is a reach seaweed extract in minerals and organic matter 35-40% (table 2), whereas moringa leaf extract contains many hormones auxins, gibberellin proteins, sugar, and many macro and micronutrients as K, Mg, P, Ca, Fe and more (Abd El-Mageed *et al.*, 2017, Yaseen *et al.*, 2023, Yaseen and Takacs-Hajos, 2022a). This result agrees with the result on lettuce by Yaseen and Takacs-Hajos (2022b), where they found greater physical parameters spraying MLE on different lettuce varieties. Also, Mohammed and Saeid (2024) found that foliar application of 3.0 and 6.0 ml L^{-1} could significantly improve the physical and yield of broccoli. Conversely, the plants treated with Alga600 exhibited the lowest leaf area as a significant physical metric, at 5.62 and 6.40, respectively, followed by the plants treated with H-made biostimulants.

Table 5. Fruit diameter, length, and leaf area as influenced by the applied biostimulants

Biostimulants	**Fruit diameter** (cm)	**Fruit length** (cm)	**Leaf area** (cm^2)
Control (untreated**)**	5.84^{a}± 1.54	8.91^{a}± 3.06	8.73^{a}± 1.83
Alga600 (0.16g pot^{-1}**)**	5.87^{a}± 1.81	11.38^{a}± 2.77	5.62^{c}± 1.45
MLE 6% (20 mL $plant^{-1}$**)**	5.75^{a}± 1.39	10.98^{a}± 3.30	7.02^{b}± 2.06
H-made 6% **(**20mL $plant^{-1}$**)**	5.90^{b}± 1.35	5.21^{b}± 2.57	6.40^{bc}± 1.42

Duncan's multiple range test shows no significant difference (*p < 0.05*) between numbers with the same letter (a, b, c, d, e).

Similar to the physical parameters, Alga600 and MLE had a significantly greater fruit weight per plant, number of fruits per plant, and total yield compared to the H-made biostimulants. However, no statistical differences were found in comparison to the untreated plants (table 6). This might be because Alga600 and MLE contain greater mineral content and lower EC which makes more ion availability in the soil (Husson *et al.*, 2018, Niste *et al.*, 2014). Researchers believe that high salt or greater EC leads to major stress in plants as ion and osmotic stresses (Jouyban, 2012, Zia-ur-Rehman *et al.*, 2016). Other research studies have demonstrated that some biostimulants can improve soil properties, biological activities and reduce soil pH and soil exchangeable Na, and increase soil NO_3^-N, resulting in greater plant growth and nutrient absorption by plant root (Karapouloutidou and

Gasparatos, 2019).

Table 6. Fruit weight, number of fruits per plant, and fresh fruit weight are among the economic criteria that are impacted by the foliar application of biostimulants.

Biostimulants	**Fruit weight** (g plant^{-1})	**No. of fruits** (plant^{-1})	**Total yield FW** (ton donum^{-1})
Control (untreated**)**	205.13^{a}± 30.70	13.67^{a}±2.05	3.57^{a}±0.53
Alga600 (0.16g pot^{-1}**)**	232.89^{a}± 20.68	14.79^{a}±1.38	3.86^{a}±0.36
MLE 6% (20 mL plant^{-1}**)**	219.45^{a}± 19.94	14.63^{a}±1.33	3.82^{a}±0.35
H-made 6% (20mL plant^{-1}**)**	158.76^{b}± 28.50	10.58^{b}±1.90	2.76^{b}±0.50

Duncan's multiple range test shows no significant difference (*p < 0.05*) between numbers with the same letter (a, b, c, d, e).

3.5 Biostimulants influence on pigment content in chili pepper leaves

Leaf pigments show the plant's potential to provide food. Applying biostimulants Alga600 and MLE 6% increased Chlorophyll a level, however there was no significant difference between the control and biostimulant treatments (*P≤0.05*). Biostimulants, on the other hand, were able to considerably improve chlorophyll b in plant leaves when compared to the control, with plants treated with 6% MLE having the greatest chlorophyll b content, followed by Alga600 and H-made at 20.09, 17.75, and 17.43, respectively. Iqbal *et al.* (2023) have demonstrated that the increase in chlorophyll increasement under drought stress might be because of some physiological changes in the plant tissues as enhanced cell turgor and the membrane protecting the amino acid's activity, as well as the control of innumerable physio-biochemical reactions inside the leaf tissues.

Alga600 had more total chlorophyll and carotenoids than other biostimulants and the control, but no significant difference was found. In contrast to pigment content, biostimulants decreased the chlorophyll ratio. The control had a significantly higher pigment ratio (Chl a/b) followed by Alga600 at 4.40 and 3.67, respectively, while peppers treated with 6% MLE and H-made biostimulants had the lowest at 2.60 and 20.91, respectively *(P≤0.05)* see table 7.

Table 7. Chili pepper pigment content and chlorophyll ratio were impacted by the foliar application of biostimulants.

Biostimulants	**Chl a** (µg ml^{-1})	**Chl b** (µg ml^{-1})	**Total Chl** (µg ml^{-1})	**Car** (µg ml^{-1})	**Chl a/b ratio** (µg ml^{-1})
Control (untreated**)**	52.44^{a}± 5.37	12.36^{b}± 1.13	64.80^{a}± 3.79	27.45^{a}± 0.83	4.40^{a}± 0.72
Alga600 (0.16g pot^{-1}**)**	63.44^{a}± 7.32	17.75^{a}± 1.37	80.80^{a}± 6.34	32.92^{a}± 4.22	3.67^{ab}± 0.70
MLE 6% (20 mL plant^{-1}**)**	54.88^{a}± 4.73	20.09^{a}± 1.96	75.79^{a}± 3.92	29.59^{a}± 3.93	2.60^{c}±0 .55
H-made 6% (20mL plant^{-1}**)**	49.96^{a}± 5.83	17.43^{a}± 1.42	67.39^{a}± 6.43	29.97^{a}± 3.45	2.91^{bc}± 0.56

Duncan's multiple range test shows no significant difference (*p < 0.05*) between numbers with the same letter (a, b, c, d, e).

3.6 Interaction between biostimulants and treatments on the physiological and yield of peppers

The major purpose of this study was to look at how biostimulants interact with drought. Table 8 reveals that the majority of the biostimulants tested responded effectively to drought stress. However, when drought levels rose, biostimulants had less of an impact on plant drought tolerance. Among the biostimulants used, H-made and MLE had a substantial influence on fruit diameter and length under light drought stress, and this effect gradually decreased as the drought stress increased. Leaf area, on the other hand, increased considerably in the management of mild drought stress and the application of 6% MLE, whereas rising drought stress reduced the MLE's effect on leaf area.

The cost-effectiveness of yield components, fruit weight, number of fruits, and total yield in plants treated with Alga600 was 12% higher than in control plants under mild drought stress, but no significant difference was seen. Similarly, plants under moderate drought stress increased fruit weight, number of fruits, and total yield by 7% after applying Alga600 and 6%MLE, compared to untreated plants, but H-made biostimulants reduced their influence by -11%, -36%, and -36%, respectively. Among the biostimulants, 6%MLE showed a beneficial interaction with moderate and severe drought stress for yield components.

Table 8. Interaction of biostimulants and drought stress on physiological and yield component in chili peppers.

Treatments / Biostimulants		Fruit diameter (cm)	Fruit length (cm)	Leaf area (cm^2)	Fruit weight (g $plant^{-1}$)	No. of fruits ($plant^{-1}$)	Total yield FW (ton $donum^{-1}$)
T1	Control	6.64^{ab}	9.64^{abcde}	10.51^{a}	212.60^{abc}	14.17^{abc}	3.70^{abc}
	Alga600	6.75^{ab}	13.19^{ab}	7.33^{bcd}	237.54^{a}	15.83^{a}	4.13^{a}
	MLE	6.63^{ab}	14.01^{a}	8.86^{ab}	225.54^{ab}	15.03^{ab}	3.92^{ab}
	H-made	7.63^{a}	6.49^{def}	7.75^{bcd}	180.16^{cd}	12.01^{cd}	3.13^{cd}
T2	Control	6.58^{ab}	9.75^{abcde}	8.58^{ab}	212.68^{abc}	14.17^{abc}	3.70^{abc}
	Alga600	6.58^{ab}	11.97^{abc}	4.99^{ef}	228.05^{ab}	15.18^{ab}	3.97^{ab}
	MLE	6.42^{ab}	10.81^{abcd}	7.28^{bcd}	227.75^{ab}	15.20^{ab}	3.96^{ab}
	H-made	5.23^{bc}	3.62^{f}	5.93^{cdef}	190.12^{bcd}	9.06^{e}	2.36^{e}
T3	Control	4.30^{c}	7.33^{cdef}	7.10^{bcde}	136.00^{e}	12.67^{bcd}	3.31^{bcd}
	Alga600	4.30^{c}	9.00^{abcde}	4.55^{f}	200.07^{abcd}	13.33^{abcd}	3.48^{abcd}
	MLE	4.20^{c}	8.13^{bcdef}	5.07^{ef}	205.08^{abc}	13.67^{abc}	3.57^{abc}
	H-made	4.83^{bc}	5.53^{ef}	5.52^{ef}	160.12^{de}	10.67^{de}	3.13^{bcd}

Duncan's multiple range test shows no significant difference ($p < 0.05$) between numbers with the same letter (a, b, c, d, e).

3.7 Interaction between biostimulants and treatments on the pigment content in pepper leaves

In general, drought stress had a greater effect on chlorophyll and carotenoids in chili pepper leaves than the interaction of biostimulants and drought stress. However, biostimulants like MLE significantly enhanced chlorophyll a and b with increased drought stress. Table 9 reveals that plants subjected to mild drought stress had considerably higher chlorophyll a, but MLE and H-made biostimulants have significantly lower chlorophyll a, and no significant difference was found in the same treatment compared to the control.

Total chlorophyll, on the other hand, increased significantly in plants treated with Alga600 under mild drought, but it decreased significantly when 6%MLE was applied. Moreover, 6%MLE increased its impact with the development of drought stress. The maximum total chlorophyll was in the plants under severe drought stress at 98.03 (µg ml^{-1}), whereas the control was 68.24 (µg ml^{-1}). The combination of drought stress and biostimulants did not substantially affect carotenoids ($p < 0.05$). The carotenoids levels were highest in plants under severe drought stress treated with Alga600, and lowest in untreated plants under moderate drought stress at 35.05 (µg ml^{-1}) and 20.54 (µg ml^{-1}), respectively.

There was a big fluctuation in the interaction of drought stress and the chlorophyll ratio (Chl a/b). Alga600 in mild drought stress, control in moderate and severe drought stress, and Alga600 recorded a significant higher Chl a/b ratio, while 6%MLE and H-made recorded a significantly lower chlorophyll ratio compared to the control.

Table 9. Interaction of biostimulants and drought stress on chlorophyll, carotenoids content, and chlorophyll ratio in chili peppers.

Treatments / Biostimulants		Chl a (µg ml^{-1})	Chl b (µg ml^{-1})	Total Chl (µg ml^{-1})	Car (µg ml^{-1})	Chl a/Chl b (µg ml^{-1})
T1	Control	48.23^{bc}	15.02^{b}	63.26^{cd}	34.66^{a}	3.25^{bc}
	Alga600	80.98^{a}	19.64^{b}	100.62^{a}	31.13^{a}	4.16^{ab}
	MLE	37.36^{c}	14.99^{b}	52.356^{d}	28.69^{a}	2.49^{c}
	H-made	48.07^{bc}	15.45^{b}	63.52^{cd}	28.82^{a}	3.12^{bc}
T2	Control	51.67^{bc}	11.23^{d}	62.90^{cd}	20.54^{a}	4.65^{ab}

	Alga600	57.36^{bc}	21.13^{b}	78.49^{abc}	32.58^{a}	2.71^{c}
	MLE	56.21^{bc}	20.78^{b}	76.99^{bcd}	30.73^{a}	2.66^{c}
	H-made	47.92^{bc}	15.62^{c}	63.54^{cd}	29.05^{a}	3.09^{bc}
T3	**Control**	57.42^{bc}	10.81^{d}	68.24^{cd}	27.16^{a}	5.31^{a}
	Alga600	50.80^{bc}	12.49^{cd}	63.30^{cd}	35.05^{a}	4.14^{ab}
	MLE	71.08^{ab}	26.95^{a}	98.03^{ab}	29.35^{a}	2.66^{c}
	H-made	53.88^{bc}	21.22^{b}	75.10^{bcd}	32.04^{a}	2.51^{c}

Duncan's multiple range test shows no significant difference ($p < 0.05$) between numbers with the same letter (a, b, c, d, e).

3.8 Pearson Correlations to the measured parameters

Our findings show that the positive and negative correlation coefficients between all analyzed attributes were either 0.01 or 0.05. Table 10 shows that fruit weight had a positive and highly significant correlation at the probability of 0.05 for the fruit diameter and fruit length which gave (.354) and (.835), respectively. This was likewise true for the number of fruits and overall yield; however, this time the significance threshold was set at 0.05. The pigment contents (chlorophyll a, b, and carotenoids) correlated positively and negatively with one another. At a probability level of 0.01, chlorophyll a had a significant positive correlation coefficient with chlorophyll b, total chlorophyll, and the ratio between chlorophyll a and chlorophyll b (.430,.968, and.408), respectively, whereas a negative correlation coefficient was found with the carotenoid content (-.004). A similar finding was obtained for chlorophyll b; however, while the correlation coefficient between chlorophyll b and carotenoids was positive, no significant difference was found. Carotenoids had just a positive association with leaf area (.138), but no statistically significant connection was found, although chlorophyll ration, number of fruits, and total yield all showed negative correlation coefficients (-.145, -.072, and -.072, respectively). Total chlorophyll, on the other hand, showed a significant negative correlation with leaf area growth at -.331 at a probability of 0.05.

Table 10. Pearson correlation among the studied data and their relation to each other

	Fruit diameter	Fruit weight	Fruit length	Chl a	Chl b	Car	Total Chl	Chl a/b	Leaf area	No of fruit	Total yield
Fruit diameter	1	.257	.354*	-.087	-.050	-.091	-.088	-.160	.295	.257	.256
Fruit weight		1	.835*	.269	.124	-.071	.263	.069	.187	1.000**	1.000**
Fruit length			1	.182	.099	.011	.182	.000	.228	.835**	.835**
Chl a				1	.430**	-.004	.968**	.408*	-.271	.269	.270
Chl b					1	.125	.642**	-.604**	-.364*	.124	.124
Car						1	.031	-.145	.138	-.072	-.072
Total Chl							1	.179	-.331*	.263	.264
Chl a/b								1	.086	.069	.069
Leaf area									1	.187	.187
No of fruit										1	1.000**
Total yield											1

*Correlation is significant at the 0.05 level (2-tailed

**Correlation is significant at the 0.01 level (2-tailed).

4.0. Discussion

Today, research on how to reduce or eliminate drought stress on vegetables is a critical concern in the Middle East and other nations experiencing water scarcity and climate change. Any approaches to reducing output losses will assist these countries ensure their food supply and continue to work on agricultural sectors. This issue is not simply about food shortage and food provision, since these countries are known for their wealth, but also about protecting their lands from desertification and degradation. Many researchers and politicians know that Iraq is losing its land day after day resulting in desertification, according to (Monitor, 2022), Iraq is losing 100,1000 dunams annually due to climate change. According to research by Gaznayee *et al.* (2022), the vegetative cover in the northern part of the country (Kurdistan region) has declined by about 40% in the last two decades because of extreme drought.

It is obvious that abiotic stress such as drought, salt stress, pollution stress, heavy metal stress, and heat stress have a greater impact on peppers in the vegetative phases rather than the flowering and fruiting stages (Iqbal *et al.*, 2023, Okunlola *et al.*, 2017). This is mainly because of the capacity of *Capsicum annum* L. to recover quickly after being subjected to short-term abiotic stress (Masoumi *et al.*, 2021, Widuri *et al.*, 2020). Since chili peppers can recover from modest drought stress, one could argue that our drought stress was not as bad as it could have been for them. Constant watering is indeed necessary for producing peppers in clay loam soil under high summer temperatures above 45°C and extreme temperatures during the season changes. Research has demonstrated that heat stress diminishes a plant's capacity to produce energy through photosynthesis, since it intensifies oxidative stress, lipid peroxidation, and a decrease in chlorophyll concentration in plants (Kipp and Boyle, 2013). Our findings, shown in Figures 3.4 and 5, illustrate that nearly every economic and quality parameter related to chili peppers is negatively impacted by moderate and severe drought. Bear in mind, that this study included one additional treatment (once every four days), but due to insufficient watering and persistent heat waves in June, July, and August and a sudden reduction of air humidity (figure 6), all of the plants gradually died in the middle of the growing season. As a result, data from the remaining treatments were excluded because there was insufficient fruit and insufficient data for statistical analysis. Our data indicate that each continuous delay of one day in watering reduces the ultimate output by -5% of the overall yield.

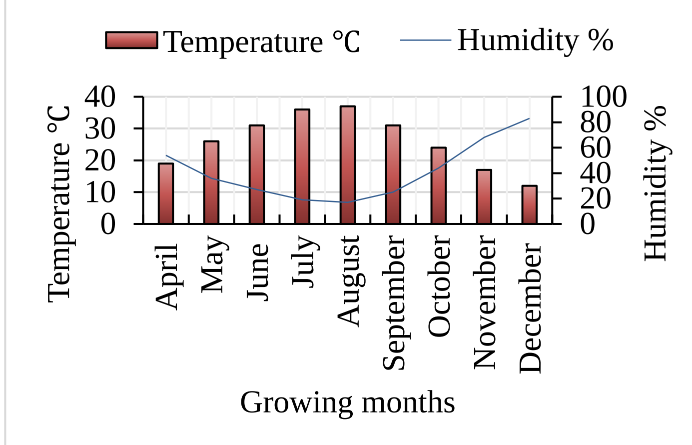

Figure 5. The average temperature and humidity during the growing season of the studied peppers.

In our research, foliar application of *Alga600 (2 mL L⁻¹), MLE 6% 20 mL plant⁻¹, Home-made biofertilizer 10% 20 mL plant⁻¹,* somehow could improve some of the measured parameters (Table 4.5.6.7), but these biostimulants might have a better result if we increase the concentration. According to the findings of this study, Alga600 and 6% MLE could boost plant tolerance to moderate and severe drought stress. This outcome was confirmed by previous studies on the use of biostimulants to alleviate plant biotic stress, such as drought and salt stress (Rakkammal *et al.*, 2023), seaweed extract (Alga) improves onions under drought stress improved yield by 9% (Ramzan and Younis, 2022), MLE also most of the physiological parameters and yield components improved by 6% and 4% of MLE in onion bulbs (Yaseen and Hájos, 2020). Improving chlorophyll b or total chlorophyll using biostimulants in this high light intensity weather condition means that the plant converts light energy to chemical energy at a greater rate. Thus, enhancing Chl b is the key to understanding the mechanisms of plant adaptation to different light intensities (Eggink *et al.*, 2001). Parađiković *et al.* (2011) found that utilizing organic plant biostimulants boosted the nutritional value of delicious yellow pepper fruit and increased the pigment content of its leaves.

It is important to notice which of the biostimulants react best with drought levels. This is because drought stress not only influences the fruit quality but also drought stress is known to induce a reduction in respiration and photosynthesis as well as damage to cell membranes mainly in higher plants (Kakar *et al.*, 2023). In this regard, the biostimulant results were subjected to statistical analysis. The results demonstrate that biostimulants were less impacted on quality improvements in pepper fruit, such as fruit diameter and fruit length when the drought was exacerbated, but more influenced by on yield components. This might be because it influences on chlorophyll content under moderate and severe drought stress levels. Crop yield relies on photosynthesis to make energy for the plants to improve or better yield (Aluko *et al.*, 2021), as in maize (Yan *et al.*, 2021), sweet peppers (Mbandlwa *et al.*, 2020). Also, water deficit is known to stimulate both non-enzymatic and enzymatic antioxidative defense mechanisms during drought stress, which eventually results in altered redox homeostasis (Ullah *et al.*, 2021). Recent research studies demonstrate a positive influence of biostimulants on abiotic stress mainly drought stress as Moringa leaf extract (MLE) improves the resistance of the soybean plant against drought stress (Ramzan and Younis, 2022).

Our results show a positive correlation between the measured parameters to the total yield. This result agrees with the result by Musa *et al.* (2023) which they found that fruit number, weight, length, stomata conductance, transpiration rate, and photosynthetic rate all correlate positively with the total production in in eggplant (*Solanum melongena* L.). Our results align with the results of studies conducted on horticulture crops and a range of other subjects (Chen and Pang, 2023, Goñi *et al.*, 2018, Jacomassi *et al.*, 2022, Rasul *et al.*, 2021).

5.0. Conclusion

Physical parameters demonstrated a positive association with one another, although chlorophyll concentration had both a positive and negative correlation coefficient with pigment content and leaf area at probability levels of 0.05 and 0.01. Increasing plant resistance to drought stress through the use of biostimulants (commercial or homemade) is a critical step towards agricultural sustainability in areas where climate change is visible, such as Iraq. Organic biostimulants, on the other hand, can protect human health and cost farmers less money to purchase or prepare on their farms while not damaging their health or the environment.

The foliar application of those three biostimulant alterations in plants, as revealed by the agronomic, physiological, and biochemical analyses of this study, offers new insights that could help improve the drought tolerance of *Capsicum annuum* L. and mitigate the adverse effects of the country's impending drought threat because of global variation. Further investigation using additional biostimulants and diverse homemade biostimulants is necessary to differentiate between all foliar treatment methods used in pepper cultivars.

To preserve our farmland's fertility and promote healthy growth rates in Iraq, we also need to learn the proper methods of irrigation to reduce the possibility of a future increase in drought. Finally, we need to start the immediate adaptation procedures recommended by the current study and additional related findings of the scholars.

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