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The Effect of Green Compost Processed Organic Fertilizer and Chlorella Microalgae Solution on Chlorophyll a, Chlorophyll b, Carotenoid and Proline Content of *Tropaeolum majus* under Drought Stress

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Environmental stresses, particularly drought, are the most critical contributors in reducing the growth of plants. The effect of processed organic fertilizer of green compost and *chlorella* microalgae solution on chlorophyll a, chlorophyll b, carotenoid and proline content of *Tropaeolum*

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majus plant under control and drought conditions were investigated. The experiments were carried out in pot with a completely randomized design with three replicates. Green manure treatment included 0%, 5% and 10% by volume of pots. Chlorella microalgae treatment had two levels in terms of zero cells and 368 million cells in each pot. Drought stress was administered based on a lack of ten-day and seventeen-day irrigation. After two months, the samples were collected and the relative content of amount of chlorophyll and carotenoids, and amino acid proline were performed. The findings revealed that green compost of 10% alone and together with chlorella microalgae significantly reduced the effects of drought stress at 5% level. Compost with 10% volumetric along with chlorella caused a significant increase in chlorophyll at control and 5% level. Tropaeolum majus plants treated with chlorella algae and 10% green compost showed the greatest levels of proline amino acid under stress.

Keywords: Chlorella microalgae; drought stress; green compost; Tropaeolum majus plant.

1. INTRODUCTION

Drought stress is one of the most important environmental stresses on agricultural products in most of the dry parts of the world. A wide range of morphological, physiological and enzymatic characteristics of the plant is affected by drought stress. Also, the mineral nutrition of the plant, especially the nutrition of less mobile elements such as phosphorus, is changed by drought [1]. The use of compost increases the growth and efficiency of plants due to the constant release of nutrients and the increase of plant efficiency in using water. Since compost has many organic materials and nutrients, its use is likely to be useful to produce any kind of agricultural and garden plants. It has been reported that plants produced using compost have better conditions in terms of quantity and quality [2]. By adding organic fertilizer to the soil, the product efficiency is improved in the short term and the soil fertility is improved in the long term [3]. Microalgae form a very diverse group of photosynthetic microorganisms. Having the power of rapid growth and survival in very difficult conditions is one of the characteristics of these resistant organisms [4]. Green waste compost, as organic amendments in the soil, is considered with advantages compared to other organic wastes due to the low risk of toxicity due to the presence of heavy metals, pollutants [5]. Proline, an amino acid with the molecular formula $C_5H_9NO_2$, is produced in the chloroplast, but it accumulates in the cytosol [6]. Stability of the natural form of proteins and protection of membrane systems [7]. Proline and glycine betaine are among the compounds that are produced in response to drought stress, but research shows that some plants do not produce enough of these compounds to deal with environmental stress [8]. Drought stress causes changes in the ratio of chlorophyll a, b and carotenoids. A decrease in the chlorophyll content of the cotton plant against drought stress has also been reported. The amount of chlorophyll in sunflower and *Vaccinium myrtillus* plants decreased significantly due to lack of water. The photosynthetic rate of the leaves of higher plants decreases with a decrease in the relative amount of water and leaf water potential [9].

1.1 Aims of Research

1) The effect of green compost and chlorella microalgae in drought stress was investigated by using the *Tropaeolum majus* plant as a model plant.
2) To investigate the interaction effect of green compost fertilizer and chlorella microalgae.
3) To assess the effect of green compost and chlorella microalgae on chlorophyll a, chlorophyll b, carotenoid and proline content of *Tropaeolum majus* plant under non-stress conditions.

2. MATERIALS AND TOOLS REQUIRED FOR TESTING

54 flowerpots - preparation of Chlorella sorokiniana microalgae from the plant physiology laboratory of Shiraz University, biology department, which was added to the soil as a solution - scale - oven - shaker - stirrer and magnet - centrifuge - spectrophotometer - water bath Warm - Ice - Vortex - glassware (Erlen, besher, graduated cylinder, test tube) - filter paper and ruler, sampler and head sampler - aluminum paper - falcon - porcelain mortar and mortar handle - hair dryer - thermometer - refrigerator and freezer - nitrogen liquid.

2.1 Research Method

This experiment was carried out from November to February 2018 in the research greenhouse of the Department of Biology, Faculty of Science, Shiraz University, and according to the investigations, the preparation of plant samples and their treatment are as follows.

2.2 Preparation of Soil and Pots for the Plants

In this research, *Tropaeolum majus* plant seedlings were prepared and transferred to pots. *Tropaeolum majus* plant voucher with code (55108) is available in every barium of Shiraz University The soil used was prepared from Eram Botanical Garden of Shiraz University. Soil was poured into each pot according to the applied treatment and according to the relative volume of the pot.

The plant was watered manually. 100 ml of water was given to the plant in every watering, which was done every two days. Also, 100 ml of *Chlorella* microalgae solution (equivalent to 36,800,000 cells) was added to the algae-treated pots.

2.3 Preparation of Green Compost

The green compost was transferred from Shiraz Municipality to the greenhouse of the Faculty of Science. This compost was prepared from plant residues.

2.4 Treatment of Green Compost and Chlorella Algae Solution to Plants

Green compost fertilizer was used at three levels of 0%, 5 and 10% of the volume of the pots. Algae solution at two levels of zero with algae three weeks after planting seedlings in the amount of 100 ml (equivalent to 368,000,000 cells) was given to the pot. In total, in this research, 54 pots with 18 treatments and three repetitions were examined, and the treatments were as follows:

C0D0A0 – C1D0A0 – C2D0A0 – C0D0A1 – C1D0A1 – C2D0A1 – C0D1A0 – C1D1A0 – C2D1A0 – C0D1A1 – C1D1A1 – C2D1A1 – C0D2A0 – C1D2A0 – C2D2A0 – C0D2A1 – C1D2A1 – C2D2A1

C0:green compost zero percent, C1: green compost 5 percent by volume, C2: green compost 10 percent by volume.
D0:zero drought, D1: low drought (ten days), D2: high drought (seventeen days)
A0: without algae, A1: with algae

2.5 Cultivation of Microalgae in Liquid Culture Medium

To prepare the liquid culture medium, Bolds Basal culture medium was used according to Table 2. In order to prepare the liquid culture medium, first stock was prepared from each of the materials in (Table 2). In this way, separate the materials of rows 1 to 7, the materials of rows 8 and 9 together, the materials of rows 10 and 11 together, the materials of rows 12 to 16 together in the amount mentioned. In the table, 250 ml of distilled water was added to the containers and autoclaved at a temperature of 121 degrees Celsius and a pressure of 15 atmospheres and kept at a temperature of 4 degrees. Then, to prepare the liquid culture medium, 10 ml of each of the 250 ml stocks were taken and made up to one liter with sterile distilled water.

Table 1. Characteristics of green compost

Measurement parameter	The amount of
Organic materials(%)	38/70
Organic carbon (%)	22/44
Total nitrogen content(%)	1/37
carbon to nitrogen ratio (C/N)	16/37
Electrical conductivity (ds/m)	4/59
pH	8/50
germination index(%)	90
K_2O (٪)	2/04
PO_4^{-3} (٪)	0/35
Ammonium to nitrate ratio	0/77

Table 2. The elements present in the stock of Bolds Basal culture medium, 10 ml of the stock is added to the volume of one liter of water and used

	Substances	Concentration(g/250ml)
1	$NaNo_3$	6.2500
2	$CaCl_2.2H_2o$	0.6250
3	$MgSo_4.7H_2o$	1.8750
4	K_2Hpo_4	1.8750
5	KH_2Po_4	4.3750
6	NaCl	0.6250
7	H_3Bo_4	0.2870
8	EDTA	1.2500
9	KOH	0.7700
10	$FeCl_3$	0.0135
11	H_2So_4	0.0250
12	$ZnSo_4.7H_2o$	0.2250
13	$MnCl_2.4H_2o$	0.0375
14	MoO_3	0.0175
15	$CuSo_4.5H_2o$	0.0375
16	$Co(No_3)_2.6H_2o$	0.0125

2.6 Preparing Pots for Treatment

From the 54 prepared pots, 18 pots are selected and 1650 grams of clay loam soil and 20 grams of perlite are mixed together to achieve a specific volume of the pot. 18 pots containing 5% green compost are mixed with 1568 grams of clay loam, 44 grams of green compost and 20 grams of perlite and added to the pots. 18 pots containing 10% green compost in the amount of 1475 grams of clay loam soil, 90 grams of green compost and 20 grams of perlite are mixed together and poured into the pot. Then, the prepared seedlings of the *Tropaeolum majus* plant were placed in all the pots and marked with a special wooden index (Fig. 1). The arrangement of the pots was done in the research greenhouse of the Faculty of Science of Shiraz University using a completely random design method. Irrigation was carried out 3 times a week at the rate of 100 ml. and *Tropaeolum majus* seedlings were kept in a greenhouse with a minimum temperature of 15 and a maximum of 25 degrees Celsius and soft light. All the pots were placed under the same environmental conditions.

2.7 Characteristics of the Soil in the Pot

Table 3. Characteristics of the soil used

Electrical conductivity (EC) (ds/m)	1/42
Total saturated acidity (pH of paste)	7/31
The percentage of neutralizing substances (T.N.V)	50/52
Organic carbon (%)	0/47
Saturation percentage S.P))	51/00
Nitrogen percentage (%)	0/02
Phosphorus (ppm)	38/20
potassium (ppm)	534/00
Clay percentage (%)	27/30
Silt percentage (%)	49/00
percentage of sand (%)	23/70
copper (ppm)	1/67
Manganese (ppm)	2/53
iron (ppm)	3/90
zinc (ppm)	4/62

2.8 How to Apply Treatments in Pots

After almost two months, the *Tropaeolum majus* plants grew in new conditions (Fig. 2). Based on the pre-experiment that was done on the edible laden plant, the drought stress level of 10 and 17 days was selected. 18 pots had no drought stress. 18 pots had low stress for 10 days and 18 pots had high stress for 17 days. Drought stress started on January 26, 2018, and plants with 10-day stress were collected on February 6, and plants with 17-day stress were collected on February 12, along with non-stressed plants. The shoot weight was measured by a precision scale (Fig. 3) and then the plant shoots were frozen by liquid nitrogen.

2.9 Measurement of Chlorophyll and Carotenoid Content

The amount of chlorophyll and carotenoid was measured using Arnon's method [10]. First, 100 mg of leaf tissue was removed and the leaf pieces were completely rubbed with 80% acetone and then their volume was increased to 10 ml. The resulting solutions were centrifuged at 3000 rpm for 15 minutes. The above solution was used to measure chlorophyll and carotenoid. For this purpose, the absorbance of the solution was measured by a spectrophotometer (Shimadzu uv-100A) made in Germany, which was blanked with 80% acetone, at 645 and 663 nm wavelengths for chlorophyll a and b and at 470 nm wavelength for carotenoid [11]. The following formula was used to calculate the amount of chlorophyll and carotenoid in the leaves. Formulas V is the final volume of the solution in milliliters [10]. Three replications were considered for each treatment. The amount of all pigments was reported in micrograms per milligram of leaf tissue.

Chl a = (11.75*A663-2.35 A645)* V/mg leaf weight

Chl b = (18.61*A645-3.96 A663)* V/mg leaf weight

Carotenoid Conten= [(1000A470- 2.27 Chl a – 81.4 Chl b)/227]* V/mg leaf weight

2.10 Proline Measurement

2.10.1 Preparation of solutions for testing proline

A: Preparation of 20 ml of 6M phosphoric acid

To prepare 6 M phosphoric acid, pour 8.13 ml of 85% phosphoric acid with a density of 1.71 into a graduated cylinder and then add distilled water to the volume of 20 ml.

B: preparation of sulfosalicylic acid 3%

Dissolve 3 grams of salicylic acid powder in distilled water and make it to 100 ml.

C: Ninhydrin acid reagent

To prepare the ninhydrin reagent, add 1.25 grams of ninhydrin in 50 ml of a solution containing 30 ml of pure glacial acetic acid and 20 ml of 6 M phosphoric acid and stir it using a magnet on the stirrer. They were stirred until the sediments were completely dissolved and then cooled at laboratory temperature.

D: Toluene

E: Preparation of proline standard solution

We dissolved 100 mg of proline in distilled water and brought it to a volume of 100 ml. Then, from this solution, we made standards of 0, 8, 16, 12 and 20 mg per liter of proline. In this way, zero, 2, 4, 6, 8 and 10 ml of the standard 100 mg/ml of proline were taken and made up to 50 ml with distilled water.

2.11 Proline Test Method

We weighed 50/g of plant sample and poured it into the mortar and added 4 cc of sulphosalicylic acid to it and grinded it until a uniform solution was obtained. Then we pour the samples into the Falcon and place them in the centrifuge, and for 10 minutes at 1500 rpm, the upper solution was accurately separated. In this case, this extract was used as the base extract for measuring proline instead of the centrifuge. After filtering the samples, pour 2 ml of filtered extract with 2 ml of ninhydrin reagent and 2 ml of pure glacial acetic acid into the test tube and mix well. Place the test tubes in a 100°C water bath for one hour. Then we transferred the samples to an ice container to cool down and the reaction ends, then they were transferred to room temperature, then 4 ml of toluene was added to the contents of the test tube and mixed vigorously for 30 seconds using a vortex machine. This action caused the contents of the tube to become 2 phases. After a period of 20 minutes, the optical absorbance of the upper solution was read at a wavelength of 520 nm and calculated using the standard curve of proline concentration in the solution. Finally, the

amount of proline was calculated based on micromoles per gram of fresh weight of the plant sample. The amount of light absorption at the wavelength of 520 nm was measured by a spectrophotometer (Shimadzu uv-100A) made in Germany. The standard graph was drawn by different concentrations of proline using Excel 2013 software.

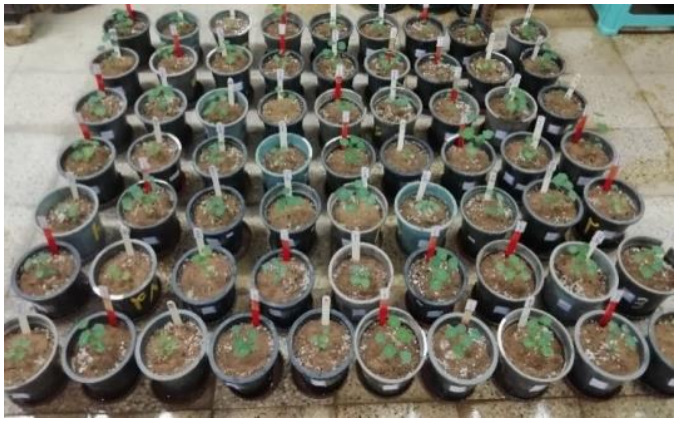

Fig. 1. How to arrange the pots in the greenhouse

Fig. 2. *Tropaeolum majus* plant after almost two months

Fig. 3. Measuring the weight of the samples

3. RESULTS AND DISCUSSION

3.1 Effects of Drought Stress, Green Compost, Chlorella Microalgae Solution on Chlorophyll a Content of *Tropaeolum majus* Plant

As can be seen in (Fig. 4) the effect of green compost processed organic fertilizer, chlorella microalgae solution and drought stress on the amount of chlorophyll a content of *Tropaeolum majus* plant. Chlorophyll a compared to control treatment. Also, no significant difference was observed in the amount of chlorophyll a content compared to the control treatment under ten days of drought stress. Also, treatments under high drought stress for seventeen days (C2D2A1, C0D2A1, C2D2A0, C1D2A0)) caused a significant increase in the level of 5% of the amount of chlorophyll a in the aerial part of the *Tropaeolum majus* plant.

3.2 Effects of Drought Stress, Green Compost and Chlorella Microalgae Solution on Chlorophyll b Content in *Tropaeolum majus* Plant

As shown in (Fig. 5) the effect of processed organic fertilizer, green compost and chlorella microalgae solution and drought stress on the content of chlorophyll b in the aerial part of the *Tropaeolum majus* plant, based on the results obtained, there is a significant difference in all treatments. The condition of the witness was not observed.

3.3 Effects of Drought Stress, Green Compost and Chlorella Microalgae Solution on Carotenoid Content in *Tropaeolum majus* Plant

As can be seen in (Fig. 6) the effect of green compost processed organic fertilizer, chlorella microalgae solution and drought stress on the carotenoid content of the aerial part of the *Tropaeolum majus* plant, mild and severe drought stress causes a significant increase in the carotenoid content in the aerial part of the *Tropaeolum majus* plant compared to the conditions witnessed. According to the obtained observations, other treatments were not significantly different from the control conditions.

3.4 Investigating the Effects of Drought Stress, Green Compost and Chlorella Microalga Solution, Chlorophyll a, Chlorophyll b, Carotenoid

Chlorophyll index in the conditions of environmental stresses such as drought and salinity is considered a suitable index to evaluate plant resistance to stress [12]. Various studies have shown that drought stress increases the production of reactive oxygen species (ROS) leading to peroxidation and as a result the decomposition of photocentric pigments [13]. On the other hand, the decrease in chlorophyll content under drought stress can be due to the change in nitrogen metabolism in relation to the production of substances such as proline, which

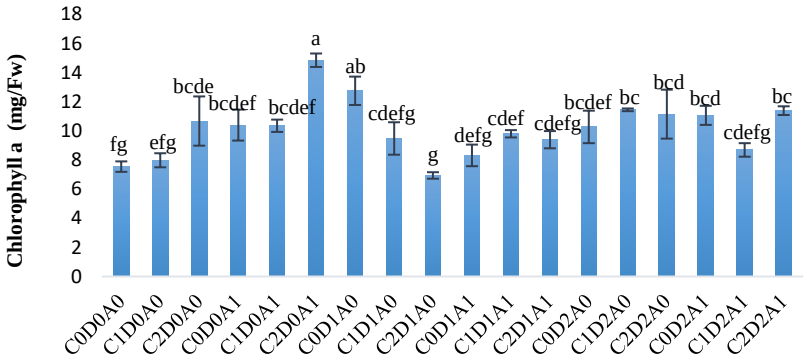

Fig. 4. The effect of green compost processed organic fertilizer, chlorella microalgae solution and dry matter on the content of chlorophyll a in *Tropaeolum majus* plant

Each number is the mean of three replicates ± SE. Different letters indicate significant differences at the p<0.05 level. C0: 0% green compost, C1: 5% green compost, C2: 10% green compost, D0: Zero drought, D1: Ten-day drought stress, D2: Seventeen-day drought stress, A0: No algae, A1: Chlorella algae solution. It has 368000000 cells

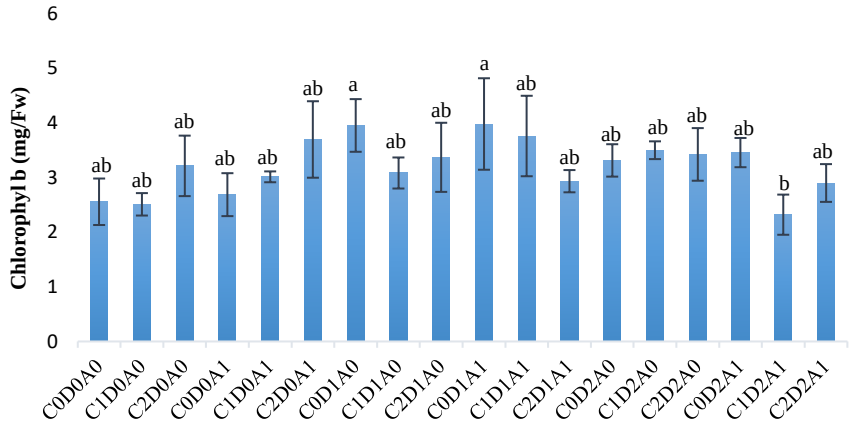

Fig. 5. The effect of green compost processed organic fertilizer, chlorella microalgae solution and dry matter on the content of chlorophyll b in *Tropaeolum majus* plant

Each number is the mean of three replicates ± SE. Different letters indicate significant differences at the p<0.05 level. C0: 0% green compost, C1: 5% green compost, C2: 10% green compost, D0: Zero drought, D1: Ten-day drought stress, D2: Seventeen-day drought stress, A0: No algae, A1: Chlorella algae solution. It has 368000000 cells

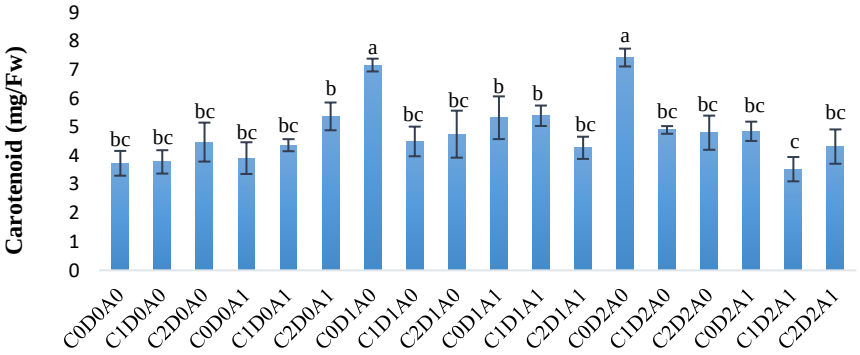

Fig. 6. The effect of processed organic fertilizer, green compost and chlorella microalgae solution and dry matter on the carotenoid content in *Tropaeolum majus* plant

Each number is the mean of three replicates ± SE. Different letters indicate significant differences at the p<0.05 level. C0: 0% green compost, C1: 5% green compost, C2: 10% green compost, D0: Zero drought, D1: Ten-day drought stress, D2: Seventeen-day drought stress, A0: No algae, A1: Chlorella algae solution. It has 368000000 cells

is used in osmotic regulation [14].Increasing the amount of proline makes glutamate, which is a precursor for the production of chlorophyll and proline, less in the production of chlorophyll [15]. In this study, it was observed that in the conditions of mild and severe stress, the levels of compost played a role in the increase and stability of chlorophyll in the growth stages of the plant, and in this way, it led to the improvement of the effects related to drought stress in *Tropaeolum majus* plant [16]. Also, the carotenoid content of the plant in dry conditions was only generally significantly different from the control [17]. These results are added to the previous researches that in drought stress, the amount of carotenoid which is considered as a support for chlorophylls against light oxidation to prevent further destruction of chlorophylls, was consistent [18].

3.4.1 Proline content

3.4.1.1 Proline standard curve

The standard curve for proline is shown in (Fig. 7).

3.5 Effects of Drought Stress, Green Compost and Chlorella Microalgae Solution on the Proline Content of *Tropaeolum majus* Plant

As shown in (Fig. 8), the effect of green compost processed organic fertilizer, chlorella microalgae solution and drought stress on the proline content of the aerial part of the *Tropaeolum majus* plant can be seen. Under conditions of mild drought stress (ten days), treatment (C1D1A1, C2D1A1) which has both compost and algae caused a significant increase in proline content compared to control conditions. Also, with increasing drought stress for seventeen days, proline content significantly increased in treatments (C1D2A0, C2D2A0, C0D2A1, C1D2A1, C2D2A1, C0D2A0) compared to the control.

3.6 Investigating the Effects of Drought Stress, Green Compost and Chlorella Microalgae Solution on Proline Content

The findings of the present study showed that with the increase in the intensity of drought stress, the amount of proline also increased significantly (Fig. 9). In general, it can be said that proline plays a role in maintaining osmotic potential, removing free radicals and ROS,

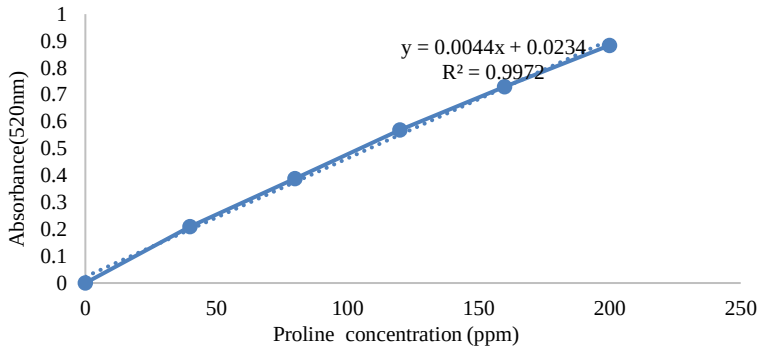

Fig. 7. According to the diagram, increasing the concentration of proline causes an increase in absorbance at 520 nm. The relationship between the amount of proline and the amount of absorption is linear

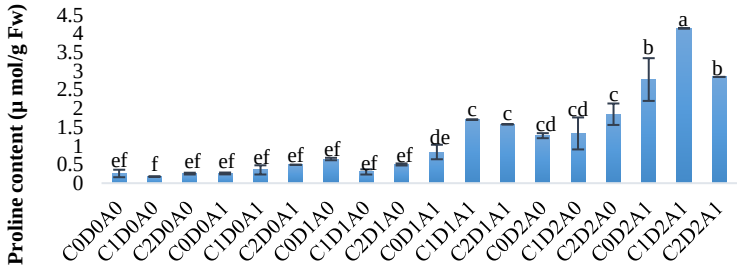

Fig. 8. The effect of green compost processed organic fertilizer, chlorella microalgae solution and drought stress on proline content in *Tropaeolum majus* plant

Each number is the average of three replicates ± SE. Different letters indicate significant differences at the p<0.05 level.: C0 green compost zero percent, C1: green compost 5 percent, C2: green compost 10 percent, D0: zero dryness D1: ten-day drought stress, D2: seventeen-day drought stress, A0: no algae, A1: chlorella algae solution with 368000000 cell

Fig. 9. The effect of drought stress, green compost and chlorella microalgae on proline (darker samples have more proline)

protecting macromolecules from denaturation, and regulating cellular pH [19]. Also, proline acts as a source of nitrogen and carbon for plants under severe stress and increases the plant's tolerance against stress [20].

4. CONCLUSION

The use of green compost and chlorella algae was useful in increasing and stabilizing chlorophyll a during drought stress, and in this way, it led to the improvement of the effects of drought stress in some treatments. Drought stress, green compost and Chlorella algae had no significant effect on the content of chlorophyll b and all carotenoids. With the increase in the amount and intensity of stress, the proline content of *Tropaeolum majus* plant increased.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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