Original Research Article

Effect of Green Compost Processed Organic Fertilizer and Chlorella Microalgae Solution on Growth, Antioxidant and Phenolic content of *Tropaeolum Majus* Under Drought Stress

Abstract
Environmental stresses, especially drought, are the most important factors in reducing growth of the plant. The effect of processed organic fertilizer of green compost and chlorella microalgae solution on growth, antioxidant properties and some other physiological characteristics of *Tropaeolum majus* plant under control and drought conditions were investigated. The experiments were performed in pot with a completely randomized design with three replications. Green manure treatment included zero, 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 applied based on lack of ten-day and seventeen-day irrigation. After two months, the samples were collected and the relative content of leaf water, fresh weight of aerial parts, phenol content and antioxidant were performed. The results showed that green compost of 10% alone and together with chlorella microalgae significantly reduced the effects of drought stress at 5% level. The use of 5% and 10% of compost with chlorella microalgae significantly increased the growth of the aerial part compared to the control. Microalgae treatments had a more positive effect on antioxidant content and phenol content than green compost in stress and control conditions. *Tropaeolum majus* plant with chlorella algae treatment with 5 and 10% green compost had the highest amount of proline amino acid under stress.

Keywords: *Chlorella Microalgae, Drought Stress, Green Compost, Tropaeolum Majus Plant.*

Introduction

Nowadays, the use of chemical fertilizers as the fastest way to compensate for the lack of nutrients in the soil has spread a lot [1]. Nitrogenous chemical fertilizers cause water and soil pollution and thus cause many diseases in humans and other creatures [2]. In order to reduce these risks, resources and institutions should be used, which in addition to providing the nutritional needs of the plant, will contribute to the sustainability of agricultural systems in the long term. Municipal waste compost as an economical and valuable organic fertilizer can be a good alternative in agriculture and organic farming [3]. The consumption of compost increases the growth and efficiency of plants due to the continuous release of nutrients and the increase of the plant's efficiency in using water. Using them brings benefits such as improving the physical structure of the soil, reducing water leaching and loss of nutrients from the soil, gradually releasing nutrients and increasing the water holding capacity in the soil [4]. 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 [5]. Iran has a dry and semi-arid climate. In this way, drought stress is one of the most important factors limiting the production of agricultural, horticultural and medicinal crops [6]. The reduction of photosynthesis is associated with the reduction of growth and yield in plants [7]. Drought is one of the environmental stresses, which after diseases is mentioned as the most important factor limiting the growth and production of agricultural plants [8]. The use of compost for soil amendment and mulching can increase the quality in the stages

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of establishment and management of plants in urban green areas. Microalgae form a very diverse group of photosynthetic microorganisms. Having the power of growth and rapid growth and viability in very difficult conditions is one of the characteristics of these resistant organisms [9]. The existence of such unique advantages is mostly attributed to the simple cell structure, the presence of a diverse range of valuable and protective metabolites, and their high physiological flexibility [10]. The plant *Tropaeolum majus* mostly grows as semi-hard seeds and both varieties one and two are available. It is seen in a wide range of shapes and colors including Beige, yellow, orange and red. This plant is strong and grows easily. This plant thrives in poor soil and dry conditions, while in rich soil, it has many leaves and few flowers.

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) Investigating the interaction effect of green compost fertilizer and chlorella microalgae was investigated.

3) Investigating the effect of green compost and chlorella microalgae on the growth of *Tropaeolum majus* plant under non-stress conditions was investigated.

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.

Research Method

This experiment was conducted 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.

Herbal Materials

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.

Preparing the soil and preparing the pots

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.

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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.

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

How to apply green compost and chlorella algae solution to pots

Green compost fertilizer was used at three levels of zero, 5 and 10% of the volume of the pots. Algae solution at two levels of zero and 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

Cultivation of microalgae in liquid culture medium

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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 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/250 ml)**
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

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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 (Figure 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 almost the same environmental conditions.

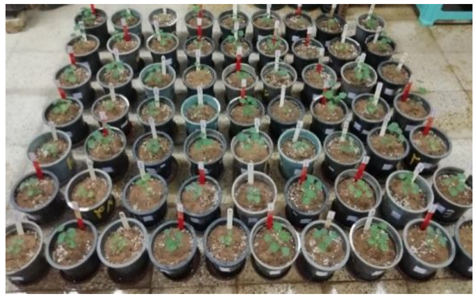

Figure 1. How to arrange the pots in the greenhouse

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	534/00

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(ppm)	
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

How to apply treatments in pots

After almost two months, the *Tropaeolum majus* plants grew in new conditions (Figure 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 (Figure 3) and then the plant shoots were frozen by liquid nitrogen.

Figure 2- *Tropaeolum majus* plant after almost two months

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Figure 3- Measuring the weight of the samples

Measurement of relative leaf water content (RWC)

Relative leaf water content (RWC) was measured according to the method of Ritchie et al. (1990). Sampling was done using leaves (one left to the last leaf without tears and breaks were selected) of all experimental treatments and their weight was immediately measured with an accurate scale. Then, the samples were cut into pieces of one square centimeter and placed in a Petri dish and distilled water was added to them (so that the leaf pieces floated in the distilled water). And we created dark and cold conditions for Petri dishes (due to no change in final weight due to evaporation or photosynthesis). That is, we put the Petri dishes in the cold room at 4 degrees Celsius in the dark for 24 hours. After 24 hours, the saturated weight of the leaves was measured and then the leaves were placed in the oven at 70°C for 48 hours. After 48 hours, the dry weight of each was measured, and then RWC was calculated by putting the numbers obtained by a scale with an accuracy of one thousandth in the following formula.

$RWC = Fw - Dw / Sw - Dw \times 100$

Fw: leaf weight immediately after sampling

Dw: dry weight of the leaf after being placed in the oven

Sw: the saturated weight of the leaf after exposure to distilled water

Measurement of antioxidants

Preparation of methanol extract of plant samples

First, we put the samples in the oven for 48 hours to dry, then we take one gram of the dried sample and put it in a porcelain mortar and grind it completely into powder and grind it with 30 ml of 100% methanol until it dissolves. until it is completely uniform, then we poured it into foil-wrapped falcons, closed the lids, and stirred it for 24 hours by Shaker. After this time, the extracts were centrifuged for 12 minutes at a speed of 4,500 rpm and the supernatant was separated and poured into other foil-wrapped falcons.

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Determining the antioxidant potential of plant samples using the DPPH method

Antioxidant potential was determined using the stable DPPH radical, according to the method described by Shimada et al., as follows [11]. In this method, the antioxidants of the sample react with DPPH free radicals and make it pale or colorless, and the reduction of DPPH color has a direct relationship with the antioxidant potential of the sample. To perform this test, methanol extract of the plant, 100% methanol, 0.004% DPPH solution in methanol and Trolox standard solution are used.

Preparation of solutions for DPPH test

A: To prepare DPPH stock solution, 0.024 grams of DPPH was weighed and poured into an aluminum-coated Erlenmeyer flask, then mixed with 100 ml of methanol and stored at minus 20 degrees Celsius. 45 ml of methanol was added to 10 ml of the above mixture, then the absorbance of the resulting solution was read at 515 nm. The amount of absorption is equal to 1.1 ± 2. If the absorption is more than 1.1, it should be diluted with methanol. 2850 microliters of the resulting solution were used in the experiments.

B: Trolox stock has a concentration of 1000 µM. Different concentrations (zero, 25, 50, 100, 200, 300, 400, 500, 600, 800 micromolar) were prepared from this stock and 150 microliters were used in the experiments.

Measurement of plant phenolic compounds

Preparing solutions to measure phenolic compounds

Phenolic compounds of plant extracts were determined using the Folin-Ciocalteu reagent according to the method of Kim et al. (2007). Gallic acid solution of 200 µg/ml was used as a standard.

A: Preparation of Folin stock: 45 ml of distilled water was added to 5 ml of Folin.

B: Preparation of sodium bicarbonate stock: 6 grams of sodium bicarbonate are added to the volume of 100 milliliters with distilled water.

C: Preparation of gallic acid stock: Ten milliliters of 50% methanol was added to 002/gram of gallic acid.

Statistical analysis of data

In the current research, a completely random statistical design was used in factorial format. The absorbed numbers were read using Spekol spectrophotometer, Analytic Jena, Germany. SPSS software was used to analyze the numbers, and finally, Duncan's test was used to compare the data considering the significance level of $p<0.05$.

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Results and discussion

The effects of drought stress, green compost and chlorella microalgae solution on RWC in *Tropaeolum majus* plant

As can be seen in (Figure 4), the effect of green compost processed organic fertilizer, chlorella microalgae solution and drought stress on the amount of RWC in *Tropaeolum majus* plant, the plants treated with green compost 10% by volume of the pot (C2) in all conditions of low stress, high stress and Without stress, no significant difference was observed in the amount of RWC compared to the control treatment conditions. Low and high drought stress only had a significant decrease at the level of 5% compared to the control. Low and high compost alone did not have a significant effect on RWC.

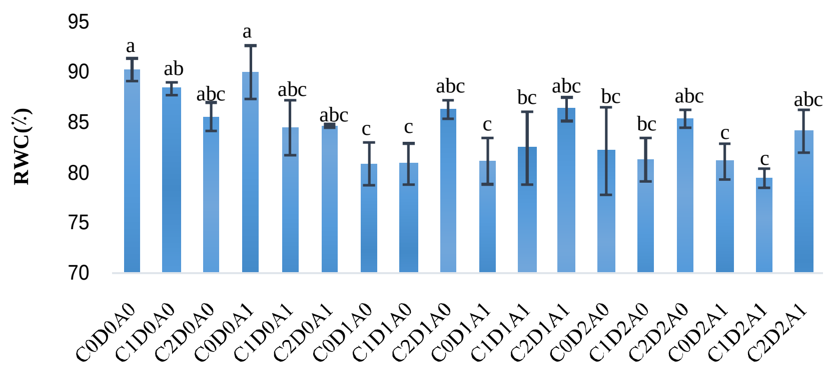

Figure 4- The effect of processed organic fertilizer, green compost, chlorella microalgae solution and dryness on the amount of (RWC) in *Tropaeolum majus* plant. Each number is the mean of three replicates ± SE. Different letters indicate significant differences at the $p<0.05$ level. : ten-day drought stress, D2: seventeen-day drought stress, A0: no algae, A1: Chlorella algae solution with 368000000 cells

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Investigating the effects of drought stress, green compost and chlorella microalgae solution on RWC (relative leaf water content) of *Tropaeolum majus* plant.

Leaf RWC is an important index in measuring leaf water status compared to other water potential parameters under drought stress conditions. During plant growth and development, drought stress reduces the amount of RWC.

Leaves tend to significantly decrease in RWC and leaf water potential when they experience dryness [5]. Also, in their studies, the researchers stated that the reduction of root growth and activity and the increase of evaporation and transpiration from plants are effective factors in reducing the relative water content in the face of drought stress [12]. The results of the present research in relation to the relative water content of the leaves were consistent with the results of Nautiyal et al [13]. regarding the physiological symptoms of soil moisture deficiency and the decrease in the relative water content of the leaves and the increase in the intensity of drought stress [13]. With the increase in the intensity of drought stress, the water absorption conditions become more difficult for plants, and as a result, the amount of water in the plant cells decreases, and the decrease in the relative water content causes a negative effect on the division of the cells and the growth and development of the plant [14]. Also, we can mention the positive effect of green compost fertilizer of ten volume percent both alone and with chlorella algae in reducing the drought stress on the plant (Figure 5).

Figure 5- The effect of ten percent green compost in severe drought stress conditions on the amount of RWC of *Tropaeolum majus* plant under control conditions (right side) and severe drought stress conditions of seventeen days (left side)

The effects of drought stress, green compost and chlorella microalgae solution on the fresh weight of the stem of *Tropaeolum majus* plant

As can be seen in (Figure 6), the effect of green compost processed organic fertilizer, chlorella microalgae solution and drought stress on the growth rate of the stem in the edible laden plant, the use of each algae and compost treatment alone in stress-free conditions causes a significant difference in the growth of the laden plant. It was not edible. But the simultaneous use of 10% by volume green compost and chlorella microalgae solution as well as 5% by volume green compost and chlorella microalgae solution in stress-free conditions caused a significant increase in plant growth compared to the control and other treatments. Also, the ten-day drought stress has caused a significant decrease in the level of 5% of the growth of aerial parts of the *Tropaeolum majus* plant compared to the control conditions.

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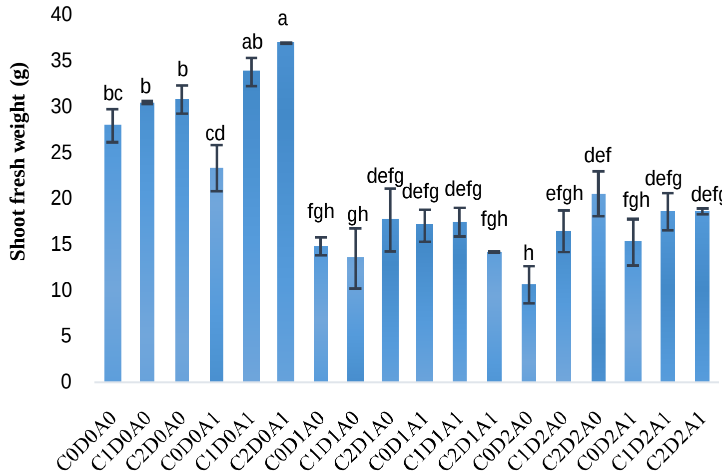

Figure 6 - The effect of processed organic fertilizer, green compost, chlorella microalgae solution and dry matter on the fresh weight of the stem of the *Tropaeolum majus* plant. Each number is the average of three replicates ± SE. Different letters indicate significant differences at the $p<0.05$ level.: 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 cells

Investigating the effects of drought stress, green compost and chlorella microalgae solution on the fresh weight of the stem of *Tropaeolum majus* plant

The present study was consistent with the results of Ros et al [15]. that the composition of green compost had a significant effect on the growth of melon plants and the best result was obtained with compost [15].

It should also be mentioned that due to the addition of chlorella algae solution to two levels of green compost treatment, there was a significant increase in the growth of the *Tropaeolum majus* plant in non-stressed conditions compared to the control treatment (Figure 7). Algal extract has a beneficial effect on plant growth due to its growth hormones such as cytokinin, indole acetic acid, gibberellin and elements such as zinc, copper, cobalt, manganese, nickel, vitamins and amino acids. Ten-day drought stress caused a significant decrease in the growth of the *Tropaeolum majus* plant, and it was consistent with the studies that observed that water stress caused a significant decrease in fresh and dry weight of mint [16].

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Figure 7- The pot of *Tropaeolum majus* plant with chlorella algae treatment

The effects of drought stress, green compost and chlorella microalgae solution on the phenolic content of *Tropaeolum majus* plant

As can be seen in (Figure 8), the effect of green compost processed organic fertilizer, chlorella microalgae solution and drought stress on the phenolic content of the aerial part of the *Tropaeolum majus* plant, the treatments (C2D0A1, C2D1A0, C0D1A1, C2D1A1) have a significant increase at the level of 5% compared to the control.

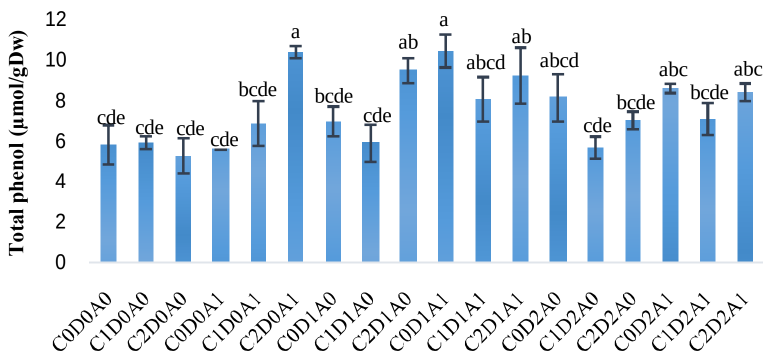

Figure 8- The effect of processed organic fertilizer, green compost, chlorella microalgae solution, and dry matter on the content of phenolic compounds in the *Tropaeolum majus* plant. Each number is the average of three replicates ± SE. Different letters indicate significant differences at the $p<0.05$ 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 cells

The effects of drought stress, green compost and chlorella microalgae solution on the antioxidant content of *Tropaeolum majus* plant

As shown in (figure 9), the effect of green compost processed organic fertilizer, chlorella microalgae solution and drought stress on the antioxidant content of the aerial part of the

Tropaeolum majus plant can be seen. Treatments containing algae (C0D0A1, C1D0A1, C2D0A1) in non-stressed conditions caused a significant increase in antioxidant content compared to control conditions. Also, mild drought (ten days) (C0D1A0, C2D1A0, C0D1A1, C1D1A1, C2D1A1, C1D1A0) treatments caused a significant increase in antioxidant content compared to control conditions. Also, in severe drought stress (seventeen days), the treatments (C0D2A0, C0D2A1, C1D2A1, C2D2A1, C1D2A0, C2D2A0) caused a significant increase in the level of 5% of antioxidant content compared to control conditions. Chlorella algae also caused a significant increase in antioxidants compared to the control.

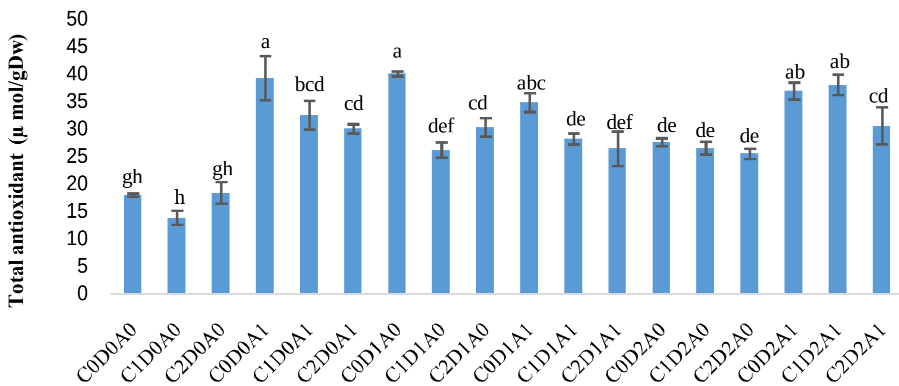

Figure 9- The effect of processed organic fertilizer of green compost and chlorella microalgae solution and dry matter on the antioxidant content of *Tropaeolum majus* plant. Each number is the average of three replicates ± SE. Different letters indicate significant differences at the p<0.05 level.: 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 cells

Investigating the effects of drought stress, green compost and chlorella microalgae solution on phenol and antioxidant content

Antioxidants are compounds that effectively and in different ways prevent the reaction of free radicals with oxygen and active nitrogen with biomolecules such as (protein, DNA) and lead to reducing damage to cells. Diphenyl picrine hydrazyl radical (DPPH) is a free radical that is widely studied for scavenging free radicals (Schutz & Fangmeier, 2001). Also, phenolic compounds include a large group of secondary metabolites, which include many cyclic compounds such as phenols, flavones, flavonoids, tannins, and lignins, and even cyclic amino acids, such as tryptophan, tyrosine, and They include proline. These compounds have many ecological and physiological roles such as defense and antioxidant roles (Andre et al., 2009). It seems that treatments with algae compared to green compost in stressed and non-stressed conditions caused a more positive effect in increasing the amount of antioxidants and phenol content in the *Tropaeolum majus* plant compared to the control treatment (Figure 10). These results were consistent with the research of Babaian et al [6] who showed that under drought stress, the phenolic compounds in this plant increase [6]. In the present study, the increase in the amount of phenolic compounds due to the increase in drought stress can be directly related to their antioxidant capacity [17]. According to the findings of Paris (2007), the main antioxidant activity of algae is due to the presence of polyphenols [18].

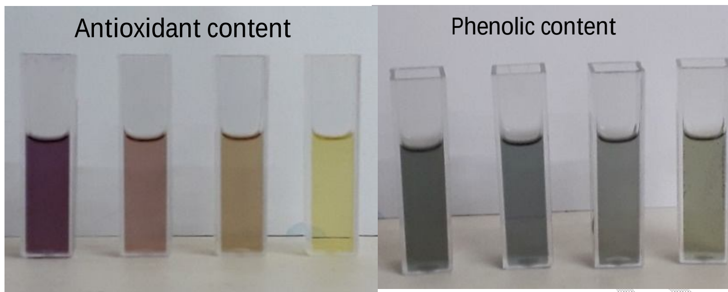

Figure 10- The effect of drought stress, green compost and chlorella microalgae on the content of phenol and antioxidant with the increase in the amount of phenol and antioxidant, the color intensity becomes more and more colorful.

Conclusion

- In general, drought stress reduces the relative water content of the leaves in the plant. It can also be mentioned the positive effect of green compost fertilizer of ten percent by volume in reducing the drought stress on the plant and maintaining the relative content of leaf water in the plant.
- The use of green compost fertilizer 5 and 10 percent by volume along with chlorella algae solution caused a significant increase in the growth of edible laden plant.
- Treatments with micro-algae had a more positive effect on the amount of antioxidants and phenol content in some places compared to green compost under stress and control conditions.

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