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Effect of some medicinal and algal extracts on some vegetative and biochemical growth characteristics of sunflower plants under water stress conditions

Mohamed Abdel-Aal Amin[1], Abo-Elela E.G.[2] and Hamad M.M. and Ismail A.O.A.[2]

[1]Department of Botany and Microbiology, Faculty of Science, Al-Azhar University, Nasr City, Cairo, Egypt.
[2]Soils, Water and Environment Research Institute, Agriculture Research Center, Giza, Egypt

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ABSTRACT

To investigate the effect of drought stress in presence or absence algal or medicinal plant extract on shoot and root length, pigments, phenol, proline content and enzymatic responses of *Helianthus annuus* L Var. Sakha 53 a field experiment based on the completely randomized design was conducted. The results showed the important role of olea extracts and *Salix alba* leaves extract in decreased the harmful effect of drought stress on growth characters (shoot and root lengths and fresh weight of shoot and root). *Spirulina platensis* extracts caused significant improved of chlorophyll content, total soluble carbohydrate, phenol and protein compared with the stress sample. *Salix alba* and *Psidium guajava* leaves extracts caused significant increase in total soluble carbohydrate, phenol and protein. Algal and medicinal plants extracts shows significant decrease in proline and antioxidant enzymes content. These results confirm the greater role these treatments in drop the harmful effect of drought stress on sunflower plants.

Keywords: Sunflower, drought stress, algal, medicinal plant, vegetative, biochemical growth characteristics.

1. Introduction

Water availability is one of the main environmental factors driving crop yield, since water is an essential component in processes like photosynthesis, transpiration, and maintenance of carbon dioxide permeability in the leaf mesophyll (Taiz and Zeiger 2013)Over the past few years, many biostimulantshave been used in agriculture, including seaweed extracts and purified compounds (du Jardin, 2015). However, microalgae, as a prospective source of plant biostimulants, have received insufficient attention (Farid *et al*., 2019). Microalgae are an excellent photosynthetic biofuel, in addition to being the largest oxygen-producing species in the world, which are essential to the ecosystem's ecological functions and environmental sustainability. Hence, microalgae may potentially be a viable option to improve and preserve crops in precision agriculture. Algae comprise active compounds, such as enzymes, free and organic amino acids, and phytohormones, in addition to secondary bioactive metabolites, vitamin precursors, and vitamins (Dineshkumar *et al*., 2018), essential nutrients, and plant hormone like auxins and cytokinins, which regulate plant growth (Renuka *et al*., 2018). It has been reported that microalgae polysaccharides have the ability to enhance plant growth; hence, they could potentially be utilized as biostimulants. *Chlorella vulgaris* has gained the attention of scientists because of its high protein levels and biomass, comprising over 55% dry weight (DW). Its high carbohydrate (15%–55% DW), lipid (5%–40% DW), and protein concentrations provide further value as feed for animals, in human cosmetics and nutrition, as well as a biostimulant(Barone *et al*., 2018). Plants have shown various responses to microalgae treatments such as increase in growth and yield, improvement in nutrient acquisition, tolerance induction to certain stress factors and maintaining the postharvest quality (Singh *et al*., 2018). Microalgal extract applications can also mitigate the adverse effects of

Corresponding Author: Mohamed Abdel-Aal Amin, Department of Botany and Microbiology, Faculty of Science, Al-Azhar University, Nasr City, Cairo, Egypt.
E-mail: mamin7780@azhar.edu.eg

abiotic stress factors among which drought and salinity predominate (El Arroussi *et al*., 2018). In this context, El Arroussi *et al.* (2018) reported that microalgae treatment mitigated the constraints arisen from different salinity levels in tomato by increasing the antioxidant enzymatic activity, phenolic compounds, and key metabolites, such as neophytadiene, tocopherol, stigmasterol, and 2,4-ditertbutylphenol, which are considered components of the main mechanisms against oxidative stress.

Although no report has been given on the use of plant extracts of *Salix alba, Psidium guajava , Olea europaea and Punica granatum* extracts as a medicinal plant for decrease the harmful effect of water stress on plant growth and bio chemical components of sunflower plants, several extracts from other plants have been used to enhancement growth of other crops, Ikechukwu (2014) studied the efficacy of crude extracts of *Senna alata*in the improvement of vegetative and reproductive growth in *Celosia aregentea*. Different concentrations (75%, 50%, 40%, 30%, 25%, 12%, 10%, and 5%) were prepared from the 100% crude extract. Seeds of *Celosia argentea* were presoaked in these different concentrations including a control (0%) and planted out after 24 hours. Results obtained showed that seedling height, leaf area, dry weight and leaf area ratio were promoted and enhanced by presoaking seeds in the extract. At the end of the experimental period (six weeks), seedling height in 75% and 100% treatments were 109 ± 16.12 cm and 117 ± 19.32 cm, leaf area 128 ± 17.91 cm2 and 125 ± 18.12 cm2, dry weight 7.48 kg and 7.0 kg respectively. Seedlings raised from seeds presoaked in water (control) however, flowered earlier (8 weeks) than the treatments (10 weeks in 75% and 100%). Presoaking seeds of *Celosia argentea* in crude extracts of *Senna alata* before planting is recommended for optimum production of the leafy vegetable. This study was therefore conducted to screen.
The role of algal and medicinal plant extracts for helping sunflower plants to tolerance against water stress. Moreover, potential of plant extracts in improving tolerance against drought stress tolerance in differentially responding sunflower was monitored.

2. Experimental

2.1. Field study

The study was carried out during the summer season 2020 to study the effect of application of the algal and medicinal plant extracts as a alleviators of water stress. Seeds of sunflower (*Helianthus annuus* L Var. sakha 53) were supplied from Agricultural Research Centre (ARC), Agriculture Ministry, Giza, Egypt. Algal and plant tissues were extracted in sterile distilled water in a ratio 1: 200 (w/v) at 60 $^{\circ}$C for 45 min. The extracts were filtered through a filter paper and stored at 4 ºC for further experimental studies (Mikhail *et al*., 2013). The extracts were applied as a foliar application at the rate of 5 g powdered extracted materials /L after 20 and 35 days of sowing. The plant samples of sunflower were collected after 40 days of sowing to analyze their morphological characters (lengths of shoot and root, fresh weight of shoot and root) and biochemical analysis (pigments, carbohydrates, protein, phenol, proline and antioxidant enzymes contents) at 40 days after sowing.

2.2. Treatments and experimental design:

This study was carried out on loamy soil the relevant chemical and physical properties of the investigated soil are shown in Table (1).These determinations were carried out according to Kiwe (1986) and Cottenie *et al.,* (1982).

The experimental area was divided experimental unite (plots) with area of 180 m^2(12 m width and 15 m length) containing 8 groups (each group was replicated three times) representing the following treatments of both interval of irrigation and extracts : control (tap water every 7 days), water stress (tap water every 14 days), water stress in presence *Chlorella vulgaris*, *Sargassum latifolium*, *Spirulina platensis*, *Salix alba*, *Psidium guajava* , *Olea europaea* and *Punica granatum* extracts respectively. The sunflower seeds were sown on one side of the ridge, with wide of (50 cm) with 20 cm apart between the hills in Botanical Garden, Botany and Microbiology Dept., Fac. of Sci., Al- Azhar Univ., Nasr City, Cairo, Egypt. The concentration of the application treatments were chosen according to a preliminary experiment in which they caused a maximum germination percentage. Before plating all plots were manured by farmyard mature (FYM) at rate of 10 $m^3fed^{-1}$with soil preparation. At the same time or money super phosphate (15.5%P_2O_5) was applied at a rate of 100kgfed^{-1} and good mixed with the surface layer of the experimental soil N treatment amomium (33.5% N) and potassium sulphate (48% K_2O) were added as a N and K fertilizers at rates of 150 and 100 kg fed^{-1} , respectively. Both N and K

fertilizer were added in a two dosed after 20 and 30 days of sowing: Other farming practical's of sunflower plaits were carried at according to the recommendations of Agriculture Minstery of Egypt.

Table 1: Physicochemical analyses of the soil used for planting.

Parameters / Sample	pH 1:2.5 (soil: water)	EC (dS/m)	Soluble cations (meq kg^{-1})				Soluble anions (meq Kg^{-1})		
			Ca^{2+}	Mg^{2+}	K^{+}	Na^{+}	Cl^{-}	HCO_3	SO_4^{2-}
Chemical properties	7.40	1.38	4.78	1.28	0.28	97.41	98.02	1.66	4.07

Table 1:cont.

Parameters	Available macro and micro-nutrients (mg/kg)						Heavy metals (mg/kg)			
Sample	P	S	Fe	Zn	Cu	Mn	Ni	Cd	Pb	Co
Chemical properties	19.15	35.46	0.43	0.13	1.37	0.39	0.20	0.01	0.93	0.04

Physical properties	Particle size distribution %					Organic matter %	CaCO3 %
	Coarse sand	Fine sand	Silt	Clay	Texture class		
	20.0	18.1	40.6	21.3	Loamy	0.44	1.42

2.3. Growth Measurements

After 40 days of sowing of bolt shorts and five plants were taken at random by from each treatment to estimated morphological characters. Roets (hellish, cm plot) and weighted immediately after clipping and estimation of the fresh weight of shoot and root.

2.4. Biochemical analysis

2.4.1. Estimation of pigments and carotenoids

Scientists Vernon and Seely (1966) discovered a method for quantifying green plant pigments. In this way, one gram-aliquots of green tissues were weighed and cut into small pieces. The plant dyes were extracted by grinding the tissue pieces in a blender for two minutes in 100 ml of 80% acetone. The mixture was transferred quantitatively and filtered using a Buchner filter installed with what man No. 1 filter paper. The filtrate was transferred to 100 ml volumetric flask and supplemented to a volume of 100 ml with 80% acetone. The optical density of the extract was measured using Carl Zeiss spectrometer at 2 wave lengths (649 and 665 nm). These wave lengths are fall in the highest absorption field by chlorophyll "a" and chlorophyll "b". The concentration of chlorophylls a, b and their sum in the plant tissues can be calculated by the following equations:

mg. chlorophyll a/g. tissue = 11.63 (A 665) - 2.39 (A 649)
mg. chlorophyll b/g. tissue = 20.11 (A649) - 5.18 (A 665)
mg. chlorophyll a+b/g. tissue = 6.45 (A 665) + 17.72 (A 649)

With respect to carotenoid pigments, the concentration was estimated according to Lichtentahler (1987) equation:

Car. = 1000x (A470) – 1.82 Ca - 85.02 Cb\198= mg\g fresh wt.

Where (A) denotes the optical density.

2.4.2. Estimation of total soluble carbohydrates and protein

Soluble carbohydrates were measured according to the method of Umbriet *et al*., (1969). Contents of soluble proteins were estimated according to the methods of lowery *et al*., (1951).

2.4.3. Estimation of total phenolics

The total phenolic constituents were estimated by using Folin-Ciocalteu method of Daniel and George (1972). Shortly, the extract was transferred to a test tube and the volume supplemented to a volume of 3.5 ml with distilled water and oxidized by adding 250 µl of Folin-Ciocalteau reagent. After five minutes, the mixture was neutralized using1.25 ml of 20% aquatic Na_2CO_3 solution. After 40 minutes, the color absorption degree was measured at a wavelength of 725 nm against the blank solution. The total phenolate content can be calculated by means of standerd curve prepared with gallic

acid and denoted by the symbol milligrams of gallic acid equivalent (mg GAE) per gm of sample. Additional dilutions can be made if the absorbance value measured was higher than the values on the standard curve.

2.4.4. Calculation of free proline

The plant content of free proline can be estimated by Bates, *et al*., (1973) in this procedure, 0.5 gm. of dry tissues was ground in 10 ml (3%) sulfosalicylic acid; the extract was filtered with what man No.2 filter paper. A mixture of 2 mls of filtrate, 2 mls acid ninhydrine (It can be prepared by heating 1.25 gm ninhydrine in 30 mls glacial acetic acid and 20 mls 6M phosphoric acid, with shaking, until melted, then cooled) and 2 mls of glacial acetic acid in a test tube were boiled in water bath for 1 hour, then the reaction was stoped in an ice bath. Then, the tube content was extracted with 4 mls toluene, mixed strongly by a test tube stirrer for 15-20 sec. the colored upper layer extracted with toluene was separated from the aqueous solution, warmed to room temperature and the degree of color absorption at a wavelength of 520 nm by UV- colormeter (Jenway). Toluene used as a blank and proline for preparation of standard curve, the proline concentration was calculated from the standard curve on the basis of dry weight of sample as follows:

$$\text{Mg/g proline} = \frac{\text{(X) ppm X ml Extract volume}}{\text{2 x Sample dry weight x 100}}$$

2.5. Extraction and estimation of enzymes catalase, peroxidese and polyphenol oxidase.

2.5.1. Extraction

The tissue parts of plant used for calculation of antioxidant enzymes, catalase, peroxidase, polyphenol oxidase and superoxide dismutase enzymes were the terminal buds in addition to young leaves. The procedure include, 2 g of the plant buds were ground with 10 ml of phosphate buffer pH 6.8 (0.1 M), then centrifuge at 2ºC for 20 min at 20000 rpm in a cooling centrifuge. The clear supernatant (containing the enzymes) was taken as the enzymes source (MuKherjee and Choudhuri 1983).

2.5.2. Calculation of Catalase activities.

Catalase activity was calculated as mentioned before that by Aebi (1984). The reaction mixture consists of a final volume of 10 ml consisting 40 µl of the enzyme extract added to 9.96 ml oxygen waterphosphate buffer at pH 7.0 (0.16 ml of 30% hydrogen peroxide to 100 ml of 50 mM phosphate buffer). Catalase activity was calculated for the change in the degree of H_2O_2 absorbance change within 60 second by a UV- colormeter (Jenway) at 250 nm. Blank was prepared by replacing the buffer solution instead of enzyme extract. The unit of enzyme activity was estimated to be equivalent to the amount of the enzyme that reduced 50% of the hydrogen peroxide within 60 second at 25^0C.

2.5.3. Peroxidase (POX) activities

Peroxidase activity was detected by solution consists of 10 ml solution (5.8 ml of 50 mM phosphate buffer pH 7.0, 0.2 ml of the enzyme extract and 2 ml of 20 mM H_2O_2 after addition of 2 ml of 20 mM pyrogallol) the rate of increase in absorbance as pyrogallol was determined spectrophotometrically by UV- spectrophotometer (Jenway) within 60 second at 470 nm and 25^0C. The blank sample was prepared by using buffer instead of enzyme extract. In case of enzyme assay, volume at zero time was taken as blank and the activity of the enzyme / g fresh weight / hour was expressed as ($\Delta \times T\ v \times 60$ min) / ($t \times v \times$ F.Wt.) where, Δ is the difference in absorbance through the incubation period, T v is the total volume of filtrate, t is the time (minutes) of incubation with substrate and v is the total volume of filtrate taken for incubation and F.Wt. is theused fresh weight (Castillo *et al*., 1984).

2.5.4. Polyphenoloxidase (PPO) activity

The activity of PPO enzyme was calculated from the method described by Matta and Dimond (1963). The enzyme and substrate mixture consists of 1.0 ml enzyme extract, 1.0 ml of 0.2 M sodium phosphate buffer at pH 7.0, 10 ml of 0.001 M catechol ($C_6H_4(OH)_2$) and 3.0 ml distilled water. The absorbance was measured at 495 nm. The difference in optical densities through 60 seconds was recorded. PPO activities were expressed as changes in the optical density/min./g fresh weight. In each

determination, control treatment (blank) contained all chemical reagents except the enzyme extract, its recorded value was subscribed from all readings. Enzyme activity can be recorded as follow, blank was prepared by replacing the buffer solution instead of enzyme extract and the activity of the enzyme / g fresh weight / hour was expressed as (Δ x T v x 60 min) / (t x v x F.Wt.) Where, Δ is the difference in absorbance before and after incubation, T v is the total volume of filtrate, t is the time of incubation in minutes and v is the total volume of filtrate taken for incubation and F.Wt. is the used fresh weight**.**

2.5.5. Superoxide dismutase (SOD) activity

The activity of SOD was estimated according to methods described by Marklund and Marklund (1974). The solution (10 mL) consisted of 3.6 mL of distilled water, 0.1 mL of enzyme, 5.5 mL of 50 mM phosphate buffer (pH 7.8), and 0.8 mL of 3 mM pyrogallol (dissolved in 10 mM HCl). The rate of pyrogallol reduction was measured at 325 nm with UV-spectrophotometer.

At harvesting 120 days of sowing, the plants of each plot were harvest and separated into shoots and flowers the seeds of the separated flowers were air-dried, weighted and calculated as $Mgfed^{-1}$

A sample of air-dried, seeds was used to determine its content (%) of oil as described by AOAC (1995).

2.6. Statistical Analysis

Results were statistically analyzed according to Snedecor and Cochran (1982).

3. Results and Discussion

3.1. Morphological characters affected by droughts of sunflower under studied treatments.

Drought is the most important abiotic factor limiting growth, adversely affect growth and crop production. In the case of water stress in table 1 appeared significant decreased of shoot and root lengths, fresh of shoot and root of sunflower (Table, 2).

Table 2: Effects of drought stress in presence or absence bio stimulant (*Chlorella vulgaris*, *Sargassum latifolium, Spirulina platensis*, *Salix alba, Psidium guajava, Olea europaea and Punica granatum* extracts) on shoot, root lengths and fresh weight of shoot and root of sunflower plants

Treatments	Shoot length(cm)	Root length(cm)	Fresh weight of shoot(g)	Fresh weight of root(g)
Control	25.14±1.021	8.321±0.214	1.654±0.121	0.287±0.014
Stress	17.801.91	6.44±1.26	0.72±0.19	0.152± 0.017
Stress + Chlorella vulgaris	20.17±3.08	4.93±1.02	0.973±0.21	0.123± 0.046
Stress + Sargassumlatifolium	21.50±2.49	6.08±1.66	1.273±0.285	0.101± 0.026
Stress + Spirulina platensis	21.12±2.32	5.20±0.79	1.269±0.282	0.158±0.057
Stress + Salix alba	22.35±1.93	7.57±1.38	1.482±0.254	0.199± 0.101
Stress + Psidium guajava	21.05±1.86	6.30±0.95	1.305±0.493	0.185±0.069
Stress + Olea europaea	23.00±2.66	7.68±1.43	1.512±0.251	0.262±0.049
Stress + Punica granatum	21.58±1.28	6.33±1.17	1.012±0.383	0.155±0.055
LSD 5%	4.54	1.12	0.59	0.043

This decrease in growth parameters was illustrated by our previous study on common bean plants a carried to out by Ghobashy *et al.,* (2020), which found a significant decrease in growth parameters of common bean (shoot lengths and fresh and dry weight of shoot and root) in response of drought stress. Application of *Olea europaea* and *Salix alba* leaves extract caused significant increase of growth parameters. *Olea europaea* leaves extract appeared the highest improvement which were 221, 19.25, 10.0, 72.7% for shoot length, root length, fresh weight of shoots and root than control treatment, respectively. Results from the sunflower cultivation trial showed an overall enhanced growth parameter of plants subjected to *Olea europaea* leaves extract treatment at the moment of the analysis. In this concern Agbagwa *et al.,* (2003) where who investigated the efficacy of spraying seedlings of *Celosia argentea* with crude extracts of *Senna alata* and recorded tremendous success ranging from promotion

of germination, vegetative and reproductive growth. Also, Farooq, *et al*., (2017) found that foliage application of all the plant extracts (Sorghum extract, Sunflower extract, Brassica extract, Moringa extract) improved the wheat performance under drought stresses, which was visible through improvement in grain weight, grain number and grain yield. Data in Table 2 also, algal extracts caused non-significant increase in height, fresh and dry weight of shoot. The importance of algal extract in stress water effect can be correlated to improvement of glycine betaine content in treated plants. In several plant species, a positive correlation between leaf osmotic potential and glycine betaine, β-alanine betaine, and proline betaine has been observed (Rhodes and Hanson, 1993). These organic compounds are now known to also have osmoprotective effects in the cell (Ashraf and Harris 2004).

3.2. Biochemical parameters

3.2.1. Pigments contents

The chlorophyll content is one of the major factors affecting photosynthetic capacity. Data presented in this worktable 2 display that water stress induced a significant decrease in chlorophyll and carotenoid contents(mg/g FW) in the sunflower leaves as compared with other treatments (under the same conditions). The drop in chlorophyll content reported in this study may be explained by a drastic reduction in the water content of this plant. The photosynthesis rate in plants reduces with the water loss in the guard cells. This may be attributed to the reduction of photosynthetic enzymes. Results in Tables 3 confirmed that in drought conditions, the used bio stimulants especially *Sargassum latifoliumor Spirulina platensis* extracts improved the chlorophyll content compared with the stress sample (irrigation each 15 days. In this trend, Rawheya *et al*., (2008) indicated that pigment content of faba bean was also increased as a result of seaweed (*Sargassum latifolium, Halimeda opuntia* and *Ulva rigida*) foliar application. Before that, Blunden *et al*., (1996) found that the seaweed concentrate increase the overall photosynthetic accumulation efficiency of the plant. Finally, the results in Table(3) also, show that, the used bio stimulants can alleviate the injury caused by drought in sunflower plants of chlorophyll contents(mg/g fresh weigh).

Table 3: Effects of drought stress in presence or absence bio stimulant (*Chlorella vulgaris*, *Sargassum latifolium, Spirulina platensis*, *Salix alba, Psidium guajava, Olea europaea and Punica granatum* extracts) on chlorophyll contents (mg/g FW) of sunflower plants

Treatments	Chlorophylla (mg/g FW)	Chlorophyll b (mg/g FW)	Chlorophyll (a + b) (mg/g FW)	Carotenoids (mg/g FW)
Control	9.012	4.21±0.026	12.21±0.336	2.654±0.136
Stress	6.63±0.68	3.50±0.215	10.13±0154	1.28±0.025
Stress + Chlorella vulgaris	7.15±0.84	2.43±0.145	9.58±0.654	2.52±0.145
Stress + Sargassumlatifolium	12.60±0.36	5.20±0.354	17.80±0.458	3.38±0.025
Stress + Spirulina platensis	10.58±0.78	4.73±0.036	15.31±0.169	2.82±0.154
Stress + Salix alba	6.38±0.65	3.53±0.254	9.92±0.486	1.38±0.047
Stress + Psidium guajava	8.21±1.25	4.24±0.325	12.46±0.463	1.57±0.089
Stress + Olea europaea	7.56±1.01	3.88±0.215	11.45±0.254	1.43±0.075
Stress + Punica granatum	7.18±0.87	3.00±0.351	10.19±036	2.02±0.042
LSD 5%	3.012	1.012	3.215	1.521

3.2.2. Metabolites contents

As recorded in Table (4) drought stress showed significant decreased in soluble carbohydrates and protein contents (mg/g dry weight)as compared with control but *Spirulina platensis*, *Salix alba* and *Psidium guajava* leaves extracts caused significant increase in total soluble carbohydrate and protein.Proline has the highest water solubility and exists in a zwitterionic state. Proline shares this property with other compounds and is collectively referred as“compatible solutes” that are accumulated in the wide range of organisms to adjust cellular osmolarity. Our results appear significant increase in proline and phenol content in response to drought stress. The accumulation of soluble phenolics under abiotic stresses helps in the stabilization of the subcellular/non-photosynthetic membranes by

detoxifying the reactive oxygen species thus improving the osmotic adjustment (Farooq *et al*., 2009). The drought stress in presence algal and medicinal plants extracts shows significant decrease in proline content. These results confirm the greater role these treatments in drop the harmful effect of drought stress on sunflower plants. The better performance of sunflower due to exogenous application of algal and plant extracts might be attributed to the presence of phenolics (Jabranand Farooq, 2013), and ascorbate, tocopherols, iron, calcium, potassium and zeatin (Hussain *et al*., 2013) in the plant extracts which eventually enhanced the sunflower performance under stress conditions. The presence of these chemicals in the plant extracts helps in the modulation of phytohormones metabolism, improvement in water/nutrient uptake, enzyme function, photosynthesis, gene expression, signal transduction (Macias *et al*., 2007), antioxidant defence system, stomatal conductance (Bogatek and Gniazdowska, 2007).

Table 4: Effects of drought stress in presence or absence bio stimulant (*Chlorella vulgaris*, *Sargassum latifolium, Spirulina platensis*, *Salix alba, Psidium guajava, Olea europaea and Punica granatum* extracts) on carbohydrates, protein, phenol, and proline contents of sunflower plants

Treatments	Carbohydrates (mg/g Dw)	Protein (mg/g Dw)	Phenol (mg/g Dw)	Proline (mg/g Dw)
Control	16.32±1.021	3.25±0.114	0.365±0.014	1.654±0.214
Stress	6.41±2.01	2.06±0.125	0.566±0.012	2.736±0.075
Stress* + *Chlorella vulgaris	10.76±3.25	2.26±0.354	0.519±0.005	2.586±0.098
Stress* + *Sargassumlatifolium	9.09±3.45	2.37±0.145	0.411±0.001	1.910±0.024
Stress* + *Spirulina platensis	26.07±4.21	2.71±0.158	0.635±0.006	1.709±0.125
Stress* + *Salix alba	15.64±4.01	2.86±0.154	0.648±0.008	2.176±0.178
Stress* + *Psidium guajava	47.79±2.02	2.91±0.214	0.717±0.002	1.679±0.048
Stress* + *Olea europaea	12.67±1.02	2.43±0.198	0.552±0.003	2.145±0.165
Stress* + *Punica granatum	8.85±2.36	2.58±0.142	0.408±0.001	1.627±0.147
LSD 5%	6.214	0.59	0.245	0.714

1- Enzyme's activities

The results in Table (5) shows the impact of water stress on the antioxidant enzyme activities (catalase, peroxidase, superoxide dismutase and polyphenol oxidase) that participate in the scavenging of reactive oxygen species (ROS).

Table 5: Effects of drought stress in presence or absence bio stimulant (*Chlorella vulgaris*, *Sargassum latifolium, Spirulina platensis*, *Salix alba, Psidium guajava, Olea europaea and Punica granatum* extracts) on antioxidant enzymes (µg/g Fw) of sunflower plants

Treatments	Catalase (µg/g Fw)	Peroxidase (µg/g Fw)	Superoxide dismutase (µg/gFw)	Polyphenol oxidase (µg/g Fw)
Control	32.2±1.2	22.32±3.2	88.32±2.25	44.25±3.21
Stress	60±2.21	36±1.25	198±4.36	156±1.25
Stress + *Chlorella vulgaris*	54±3.25	14±1.32	148±4.32	65.4±2.02
Stress + *Sargassum latifolium*	47±1.25	27±2.21	100±5.21	76±1.02
Stress + *Spirulina platensis*	52±3.21	24±2.32	182±3.21	93.8±2.36
Stress + *Salix alba*	30±4.21	13±1.65	194±3.25	82.8±4.32
stress + *Psidium guajava*	36±1.21	23±2.32	140±3.69	72±4.36
Stress + *Olea europaea*	30±2.01	20±1.25	126±4.32	54.8±2.32
stress + *Punica granatum*	21±3.21	24±2.32	90±1.32	62±1.25
LSD 5%	10.25	6.21	12.32	15.21

The results showed a jump in catalase, peroxidase, superoxide dismutase and polyphenol oxidase activities in leaves of the apical tip of sunflower plants under drought treatment compared with control. Water stress is ultimately related to enhanced oxidative stress because of increased accumulation of

ROS, mainly O_2^- in chloroplasts, mitochondria, and peroxisomes. The induction of antioxidant enzyme activities is a general adaptation strategy which plants use to overcome oxidative stresses (Foyer and Noctor 2003). Table (5), also, shows that a bio stimulant can alleviate the injury caused by oxidative stress as drought stress result in sunflower plants. The addition of bio stimulants could greatly significantly decrease antioxidant enzymes. This may be because of bio stimulant contain many of osmoprotectants molecules, which reduces the harmful effects of water stress.

Data presented in Table (6) revealed that the important foliar application of role of bio agents especially Salix alba and Olea europaea leaves extracts, as well as Spirulina platensis extracts caused increase of seed yield this is due to decreasing the harmful effect of drought stress on growth characters and improved of chlorophyll content, total soluble carbohydrate, phenol and protein compared with the stress sample. As the study showed algal and medicinal plants extracts shows significant decrease in proline and antioxidant enzymes content.

Table 6: Effects of drought stress in presence or absence bio stimulant (*Chlorella vulgaris*, *Sargassum latifolium, Spirulina platensis*, *Salix alba, Psidium guajava , Olea europaea and Punica granatum* extracts) on seed yield of sunflower plants

Treatments	Seed yield (kg plot^{-1})	Seed yield (kg fed^{-1})
Control	49.7	1107
Stress	27.9	620
Stress + Chlorella vulgaris	28.8	640
Stress + Sargassum latifolium	37.8	840
Stress + Spirulina platensis	36.9	820
Stress+ Salix alba	33.3	740
Stress + Psidium guajava	49.5	1100
Stress + Olea europaea	31.9	710
Stress + Punica granatum	43.2	960

Conclusion

In conclusion, the present study showed that:

1- *Olea europaea* then *Salixalba* leaves extract appeared the highest improvement for shoot length, root length, fresh weight of shoots and root under stress.
2- *Sargassum latifolium* then *Spirulina platensis* extracts caused the best significant improved of chlorophyll content, total soluble carbohydrate, phenol and protein compared with the stress sample.
3- *Psidium guajava* extracts showed the highest improvement for total soluble carbohydrate, phenol and protein compared with the stress sample.
4- A jump in proline, catalase, peroxidase, superoxide dismutase and polyphenol oxidase activities in leaves of the apical tip of sunflower plants under drought treatment compared with control but, a bio stimulant can alleviate the injury caused by oxidative stress as drought stress result in sunflower plants. The addition of bio stimulants could greatly significantly decrease antioxidant enzymes.
5- In this study showed algal and medicinal plants extracts shows significant decrease in proline and antioxidant enzymes content. These results confirm the greater role these treatments in drop the harmful effect of drought stress on sunflower plants.

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