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# Seaweed extract treatment enhances vegetative growth and antioxidant parameters in water stressed *Salvia officinalis* L.

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## Abstract

The effect of seaweed extract (SWE) of *Fucus spiralis* (macroalgae) on tolerance of sage plants (*Salvia officinalis*) cultured under water deficit was studied. Plants were cultivated under three levels of water deficit: without stress (WS), moderate water deficit (MWD), severe water deficit (SWD), treated with SWE. SWE enhanced vegetative growth in sage with or without stress. The maximal shoot length was observed with application of 25% *Fucus spiralis* extract as well as leaf number. Treatments by SWE at 25% and 50% enhanced leaf area under MWD compared to control. The lipid peroxidation was low in stressed plants treated with SWE at 50%. Total phenolic content was increased in sage plants treated with 25% SWE under different growth conditions WS, MWD, and SWD. The highest total phenolic content was attained with the application of 25% *F. spiralis* extract in sage plant WS and subjected to drought stress (MWD). There was a significant enhancement of glycine betaine content (GB) but the response after application of SWE was variable. Drought stress had no significant effect on ascorbate peroxidase (APX) activity; however, plants treated by SWE at 50% and 75% increased this activity and reached 3.2 UE $min^{-1}$ mg $protein^{-1}$. Catalase activity (CAT) as well as superoxide dismutase activity (SOD) increased under drought stress; but these activities decreased after treatment by 50% SWE. The enhancements of antioxidant activity by SWE contribute to protection against peroxidation of lipids and reduce the severity of water deficit on sage plants.

## 1. Introduction

*Salvia officinalis* L. commonly known as an important medicinal plant [1]. It is largely utilized both as a part of culinary and therapeutic arrangements [2,3] as an antispasmodic, antimicrobial, and for mitigating carminative and mucolytic issues. Sage has 900 species all through the world that can be utilized as crude material for medications. Numerous *Salvia spp* are exploited as home grown tea, for enhancing nourishment and in cosmetics, perfumery and the pharmaceutical business [4]. Today, for its therapeutic, fragrant, and culinary properties, it is cultivated in temperate regions all around the world. It can be added to food stuffs providing that the concentration of thujones present in the final product does not exceed 0.5mg/kg.

The general term "drought" covers different notions. We distinguish a lack of water (drought) from a structural deficit of water (aridity). The aridity is a systematic rainfall deficit. In arid regions rainfall is lower than evapotranspiration potential. Nearly a third of the total area of the world is made up of dry lands. In contrast, drought defines a non-systematic rainfall deficit and thus is characterized by the intensity of its deviation from an average or normal value of rainfall [5]. Different quantitative elements characterize drought: the duration (intermittent or prolonged drought), the period of occurrence, the geographical extension, and the dynamics of establishment (sudden or progressive) and the time of occurrence within the crop cycle [6]. Thus, when looking at cultures of our regions, corresponds to a soil water deficit resulting in a reduction in the quantity of water absorbed by the whole plant a reduction in the quantity of water absorbed by the plant compared to the amount of

water evaporated. This difference between the water absorption and evaporation is characterized by a decrease in the efficiency that would be expected under favorable conditions and a decrease in the quality of the harvested products [7]. Investigations carried out in the past provide considerable insights into the mechanism of drought tolerance in plants at the molecular level [8]. Under severe water deficiency, cell elongation of higher plants can be inhibited by interruption of water flow from the xylem to the surrounding elongating cells [9]. Also, water deficit causes limitation to carbon dioxide uptake because of stomatal closure and thus direct inhibition of photosynthesis occurs, while the photosynthetic machinery shows some signs of down-regulation [10], and resulting the deterioration of tissue by reactive oxygen species (ROS) [11]. In stressed plants, soluble sugars usually accumulate in the vacuole for osmoregulation [11]. All plants have an antioxidant system, generally two kinds of systems: enzymatic (superoxide dismutase, SOD; catalase, CAT; ascorbate peroxidase, APX) and non-enzymatic (ascorbic acid, glutathione, phenolic compounds, alkaloids, non-protein amino acids and α-tocopherols) antioxidant defense systems [12]. However, some biochemical parameters are used to determine the effect of water deficit on plants such as malondialdehyde content (MDA) which is a breakdown product of lipid peroxidation [13]. As well, the content of low molecular weight osmolytes, including glycinebetaine, proline and other amino acids, organic acids, and polyols, are determined because it's crucial to sustain cellular functions under drought by the mechanism of osmotic adjustment which maintains water relations under osmotic stress. The osmotic potential of the cell is lowered, which attracts water into the cell and helps with the maintenance of turgor [14]. In the case of aromatic crops, drought may cause significant changes in yield and composition of some metabolites [15]. Membranes are main targets of degradative processes induced by drought. Membrane lipid content decreases after water deficit and is correlated with the inhibition of lipid biosynthesis [16].

The utilization of seaweed extract in agriculture to promote plant growth has been studied after the first commercialization in 1940 [17]. Seaweed extract are well-established organic plant biostimulants, that are used for enhancing growth and yield of various plants, by improving the mobilization of nutrients, enhancing the development of roots and increasing foliar surface by improving chlorophyll content [18]. These effects are due to the presence of hormones (plant growth regulators) in seaweed, which have an effect on plants [19]. Also, the polysaccharide of seaweed degraded to oligosaccharide has an effect on promoting the plant metabolism [20].

The main objective of our study was to test the effects of foliar application of seaweed extract obtained from the macroalgal species *Fucus spiralis*, and to evaluate some morphological and biochemical parameters in treated and untreated sage under water deficit.

## 2. Material and Methods

*2.1. Preparation of seaweed extract*

The seaweed extracts were prepared from a macroalgae species *Fucus spiralis* (Phaeophyceae). It was collected from the coastal area of Sidi Bouzid near El Jadida city (Morocco) in March. The algae were hand-picked and thoroughly washed with seawater to remove all impurities, adhering sand particles, and epiphytes. Samples were washed by tap water to remove the surface salt and then blotted to remove excess water. Fresh material was cut into small pieces of 2 cm and preserved at −20 °C until use. One kilogram of fresh seaweed material was boiled with 1 L of distilled water for 1 h and filtered through a double layered muslin cloth to remove debris. These filtrates were chosen as 100 % of seaweed concentration. The concentrations of SWE were prepared by adding distilled water 25%, 50% and 75%. In order to preserve organic matter, seaweed extracts were stored at −20 °C until use.

*2.2. Plant material preparation and treatments*

*Salvia officinalis* plants used in this work was obtained by cutting propagation. The mother plants were cultivated in a plant nursery, 23 km south-east of Marrakech. Stems with two nodes and four opposite leaves were cut from mother plants and transplanted in dimpled plates filled by peat. After 25 days, the plants were transplanted into plastic pots (1.5 L) containing agricultural soil and washed sand mixture (1:1) ratio. Experiments were carried out in a greenhouse of Faculty of sciences and techniques (Cadi Ayyad University, Marrakech) at 28 °C day/ 20 °C night for 90 days. Seaweed extracts (SWE) was applied to plants by foliar spray (2 mL / plant) at different

concentrations and under water deficit conditions (Without stress (WS) moderate water deficit (MWD) and severe water deficit (SWD)) every 5 days. The plants were divided into different lots (nine plants per treatment).

*2.3. Determination of field capacity*

We filled a pot with 1 kg of dry soil (80 °C for 48 h), and then, we poured water until saturated; after 24 h, we weighed the pot; the difference between the wet weight and dry weight means the maximum volume of water held in the soil which represents 100 % of field capacity. The weight of each pot was maintained at field capacity of water deficit treatment (1 mL of water correspond to 1 g).

*2.4. Morphological parameters*

Plant growth was measured on the basis of height of the plants (in cm), leaf area, and number of leaves. The morphological data measured at the end of the trial (day 90).

*2.5. Glycine betaine (GB) assay*

According to the method of Grieve and Grattan [21]: 0.5 g of leaves dried at 80 °C for 48 h crushed in 15 mL of distilled water. All the tubes were mechanically shaken for 24 h at 25 °C, 0.5 mL extract was mixed with 0.5 mL 2 N $H_2SO_4$ solution and shaken in ice for 60 min. After incubation, 0.2 mL potassium tri-iodide solution (containing 7.5 g iodine and 10 g potassium iodide in 100 mL 1 N $H_2SO_4$) was added. After 16 h of storage at 4 °C and centrifugation (8000×g for 15 min at 0 °C), 9 mL of dichloromethane (chilled at −10 °C) were added to the supernatant after gentle shaking by vortex. By passing a continuous stream of air for 1–2 min, two layers were separated, the upper aqueous layer was discarded, and optical density of the organic layer was recorded at 365 nm by spectrophotometer. The concentration was estimated by using a standard curve developed with different concentrations of glycine betaine (SIGMA-Aldrich® chemie Gmbh, Finland).

*2.6. Determination of malondialdehyde (MDA)*

Was measured by method described by Šavicka and Skute [22]. Lipid peroxides were extracted from 0.1 g fresh weight of leaves crushed in 0.5 mL trichloroacetic acid (TCA 0.1%). The extract was centrifuged (15,000×g for 20 min), 1 mL of supernatant was mixed with 2.5 mL of 0.5 % (w/v) TBA (in TCA 20%). A chromogen was formed after incubation of reaction mixtures at 95 °C for 30 min. After centrifugation (15000×g for 30 min) the reaction was stopped by shaking in an ice cold water bath. The absorbance of the chromogen formed was determined as TBA–MDA complex at 532 nm and 600 nm using a spectrophotometer. The amount of MDA was calculated: $\varepsilon = 155.0\ mM\ cm^{-1}$

*2.7. Estimation of total phenolic content*

50 mg of fresh weight leaves were crushed by 1 mL 80 % methanol at 4 °C. After centrifugation at 19000×g for 20 min the supernatant was used for phenol content analysis. Phenolic content of the extract was estimated by the method of Taga et al. [23]. 100 µL of aliquot sample was mixed with 2 mL 2 % $Na_2CO_3$ and allowed to stand for 2 min at room temperature. 100 µL 50 % Folin Ciocalteu's phenol reagent was added after incubation and then reaction mixture was mixed thoroughly and allowed to stand for 30 min at room temperature in the dark. Absorbance was measured at 720 nm by spectrophotometer. Gallic acid (Sigma Aldrich, Finland) was used as a standard for preparing the calibration curve. Phenolic content was expressed as gallic acid equivalent (GAE) in µg by mg of estimated dry weight after determination of humidity content of fresh sample.

*2.8. Enzyme extractions and assays*

The crude enzymatic extract was prepared following the method of Tejera et al. [24]. 100 mg fresh material was homogenized with 2 mL potassium phosphate buffer (0.1 M, pH 6) and 5 % insoluble polyvinylpyrrolidone, centrifuged at 12,000×g for 30 min. The supernatant was used for estimation of enzyme activity. The enzyme protein was determined by the method of Bradford [25] for all the enzymes for expressing the specific activity of enzymes.

*2.9. Superoxide dismutase activity (SOD)*

SOD activity was assayed according to Beyer and Fridovich [26]; the reaction mixture contained 8.3 mg $L^{-1}$ riboflavine, 2.14 g $L^{-1}$ methionine, and 67 mg $L^{-1}$ nitroblue tetrazolium salt (NBT) dissolved in 50 mM sodium phosphate buffer (pH 7.8). Four milliliters of the reaction medium was added to 200 µL enzyme extract. The mixtures were illuminated in glass test tubes by two sets of Philips 27 W fluorescent tubes in a single row. Illumination was started to initiate the reaction at 20 °C; after 3 min, the absorbance was read at 560 nm using a spectrophotometer against the blank. A percentage of inhibition (%I) was determined by the following formula:

%I= [(Ab-As)/Ab]×100

Where Ab is the absorbance of blank and As is the absorbance of sample. SOD activity was expressed in units (UE $mg^{-1}$ protein) where 1 U is defined as the amount of change in the percentage of inhibition (%I) by 50 %.

*2.10. Catalase activity*

Was determined following the method of Aebi [27] and modified by Jaleel et al. [28]. The reaction mixture contained 1.9 mL 50 mM potassium phosphate buffer (pH 7) with 10 mM $H_2O_2$, and 0.2 mL enzyme extract. The decomposition of $H_2O_2$ was followed by the decline in absorbance at 240 nm using a spectrophotometer. The enzyme activity was expressed in unit per minute per milligram protein (UE $min^{-1}$ mg $protein^{-1}$; UE=1 mM of $H_2O_2$ reduction).

*2.11. Ascorbate peroxidase activity (APX)*

APX activity was assayed according to Nakano and Asada [29]. In test tube, 1 mL potassium phosphate buffer (100 mM; pH 7), 1 mL ascorbic acid (0.5 mM), 0.1mL $H_2O_2$ (0.1 mM), added on 100µL enzyme extract. The decomposition of ascorbic acid was followed by the decline in absorbance at 290 nm using a spectrophotometer. The enzyme activity was expressed in unit per minute per milligram protein (UE $min^{-1}$ mg $protein^{-1}$; UE=1 mM of ascorbic acid reduction).

*2.12. Statistical analysis*

All data were analyzed using the SPSS statistical package (version 20.0; SPSS Inc., USA). Two-way ANOVA (factor 1, drought stress level; factor 2, SWE concentrate treatment), followed by the Student Newman–Keuls post hoc test, was used to compare differences in the means ($P<0.05$). Values are expressed as the mean±standard deviation (n=3).

## 3. Results and discussion

***Growth parameters*** These parameters have reduced under water deficit compared to the control (Figure 1). The treatment by SWE 25% without stress (WS) enhanced significantly the shoot length compared to control. Leaf numbers has increased in plants treated by SWE 25%, and 75% without stress, also we identified an improvement of leaf number in plant treated by SWE at 25% and 75% under MWD and in plants treated by 50% and 75%. Leaf area has augmented in plants without stress treated by SWE at 25% and 50% as well we detected an enhancement in plants under MWD and SWD treated by SWE at 25% and 50% compared to control.

Abiotic stresses such as drought, salinity, and temperature extremes can reduce the yield and quality of agricultural crop [30]. The response of plants to drought occurs at the physiological, cellular and molecular level. It depends on many factors, such as the species and genotype, duration and water deficit severity, age and stage of plant development, the organ, cellular and subcellular compartment types affected [31]. In *S. officinalis*, drought reduced shoot length, leaf area, and leaf number. These results are related to those of Bettaieb et al. [32] and reported that water deficit decrease plants biomass. In our study, the application of SWE enhance the vegetative growth of sage under different growth conditions (WS, MWD, SWD) this results are similar after the application of seaweed extract on other plants, like *Vinea sinensis* and *Phaseolus vulgaris* [33, 34]. Chemical components of seaweed like cytokinin, auxin, betaine and betaine-like components that affect plant growth [18].

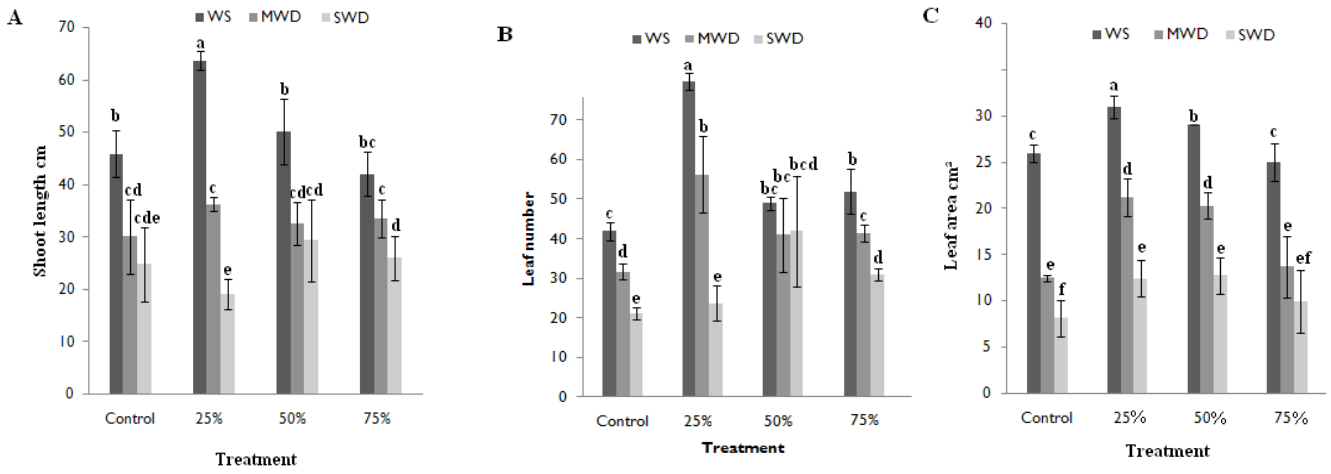


**Figure 1** The effect of water deficit on shoot length (A), leaf number (B) and leaf area (C) of *Salvia officinalis* sprayed with SWE. Results are means±SD (n=4).Different lettersfor each mean show statistically significant differences at P<0.05

***Glycine betaine (GB)*** We detected an augmentation of GB content in stressed plants (MWD and SWD) compared to control (Figure 2). However the GB content increased in plants treated by 50% SWE under WS, in addition the plants treated by 25 and 50% of SWE under SWD have a high content of GB compared to control. An improvement has observed in plants treated with 75% under MWD. GB content decreased in plant treated with 25% of SWE under WS and MWD respectively and among 50% of SWE under MWD compared to control.

Treatment by SWE enhance betaine content, generally, the osmolytes or compatible solutes are hydrophilic metabolites such as sugars, amino acids (proline and betaine), polyols (glycerol, mannitol), and other low molecular weight metabolites [35] have an important responsibility in the maintenance of the osmotic potential in the cytosol and in protein stability and cell structures [36]. Betaines are an important group of osmoprotectors with glycine betaine as the most common [37].

***Malondialdehyde (MDA)*** Water deficit has an effect on MDA accumulation (Figure 3). More the severity of stress is important, the plants accumulated more MDA. MDA content of plant WS was constant in all SWE treatment, but MDA content in the majority of plants stressed (MWD, SWD) and treated by SWE decreased against to control.

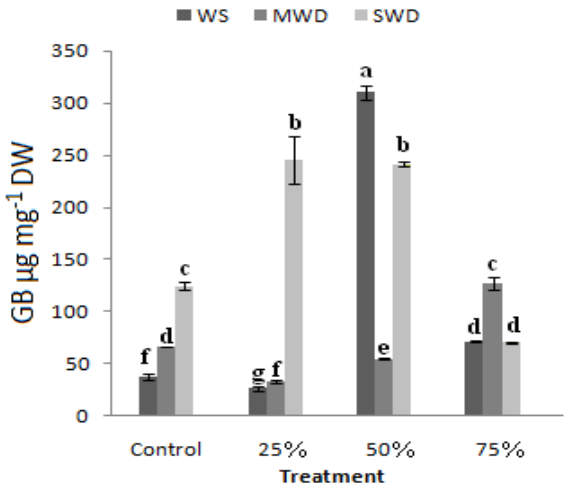


**Figure 2** The effect of water deficit on glycine betaine in plant leaves of *Salvia officinalis* sprayed with SWE. Results are means±SD (n=3).Different letters for each mean show statistically significant differences at P<0.05

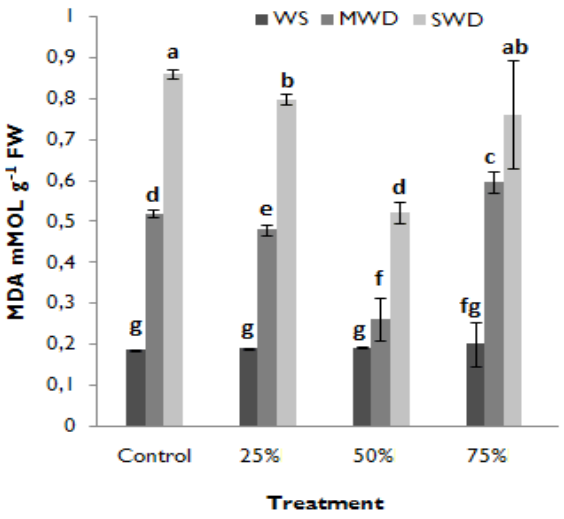


**Figure 3** The effect of water deficit on malondialdehyde in plant leaves of *Salvia officinalis* sprayed with SWE. Results are means±SD (n=3).Different letters for each mean show statistically significant differences at P<0.05

The foliar MDA content increased in the leaves of sage plants after water deficit, this is because drought-induced formation of active oxygen occurs in the chloroplasts [11]. The production of MDA was correlated to reactive oxygen species (ROS) [38]. The generation of $O2^{\bullet -}$may trigger the formation of more reactive ROS like $OH^{\bullet}$, each of which may cause peroxidation to membrane lipids and cellular weakening [39]. The treatment with seaweed extract of *F.spiralis* decreases MDA content that can indicating a tolerance of sage plant on drought stress and lower accumulation of ROS in plants.

***Total phenolic content (TPC)*** There is no a significant difference in TPC between unstressed plants and under MWD In untreated plants (Figure 4). However, TPC decreased in untreated plants under SWD against to MWD and WS plants. Treatment with SWE at 25% enhanced TPC in plants WS and under MWD, SWD compared to control. Besides TPC improved in plants under MWD treated by SWE 50% as well as plants under SWD treated with SWE 75% against to control.

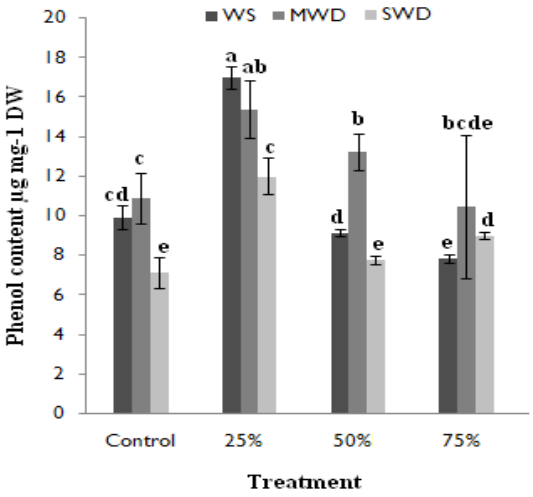


**Figure 4** The effect of water deficit on total phenolic content in plant leaves of *Salvia officinalis* sprayed with SWE. Results are means±SD (n=3).Different letters for each mean show statistically significant differences at P<0.05

The beneficial effect of seaweed extract on the tolerance of sage under drought can be explained by enhancement of phenolic content in treated plants.

Plant phenolic compounds such as flavonoids and lignin precursors are potent antioxidants [40]. However, accumulation of phenolics in plants can be induced by abiotic and biotic stresses [41]. During drought stress, active oxygen species are formed and can cause oxidative damage [42]. The toxic effects of ROS are counteracted by enzymatic as well as nonenzymatic antioxidative system such as phenolic compounds which possess an ideal structural chemistry for free radical scavenging activity [43]. phenolic compounds has a high reactivity as hydrogen or electron donors, and the ability of the polyphenol-derived radical to stabilize and delocalize the unpaired electron (chain-breaking function). Phenolic compounds chelate transition metal ions (termination of the Fenton reaction) [44, 45].

***SOD activity*** Under water stress, SOD activity enhanced relatively with severity of drought (WS, MWD, SWD) (Figure 5). But plants treated by SWE have a different response. We detected an improvement of SOD activity after treatment by SWE at 25% and 75% in plants WS as well in plants MWD treated by 75%. SOD activity decreased in plants under MWD treated with 25% of SWE and in plants under SWD treated by 50% and 75% respectively.

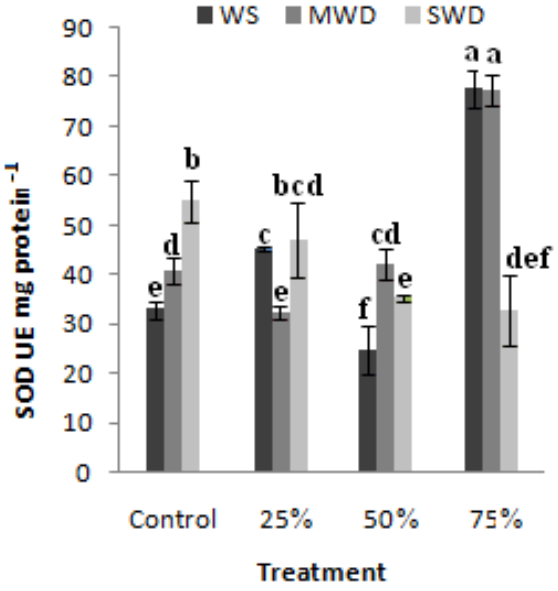


**Figure 5** The effect of water deficit on SOD activity in plant leaves of *Salvia officinalis* sprayed with sprayed with SWE. Results are means±SD (n=3).Different letters for each mean show statistically significant differences at P<0.05

SOD activity responds to water deficit differently in different experiments and species. Our results indicate that SOD increased after drought stress, however, in abiotic stresses, reactive oxygen species (ROS), such as superoxide radical, hydrogen peroxide ($H_2O_2$), and nitric oxide (NO) are produced and some antioxidant enzymes like SOD catalyzes dismutation of toxic superoxide to hydrogen peroxide [11]. The response of SOD after treatment by SWE has changed compared to control, by improving or decreasing this activity in several treatment. Zhang and Schmidt [46] reported that the application of *Ascophyllum nodosum* extract also increased superoxide dismutase activity and alleviated the decline in turf grass quality while also increasing photochemical efficiency and root viability.

***Catalase activity (CAT)*** Drought stress enhanced significantly CAT activity in untreated plants, however plants WS treated by seaweed extract 25% and 75% has a highest activity than control, its similar to activity of untreated plants under SWD (Figure 6). Activity of CAT decreased in plants under MWD treated by 25% and 50% of SWE compared to control.

***Ascorbate peroxidase activity (APX)*** Sage treated with SWE at 50% and 75% under all stress improve APX activity compared to control, in addition unstressed and MWD plants treated by 25% SWE had no a significant difference with control, but the 25% treatment increased the APX activity in plant under SWD (Figure 7).

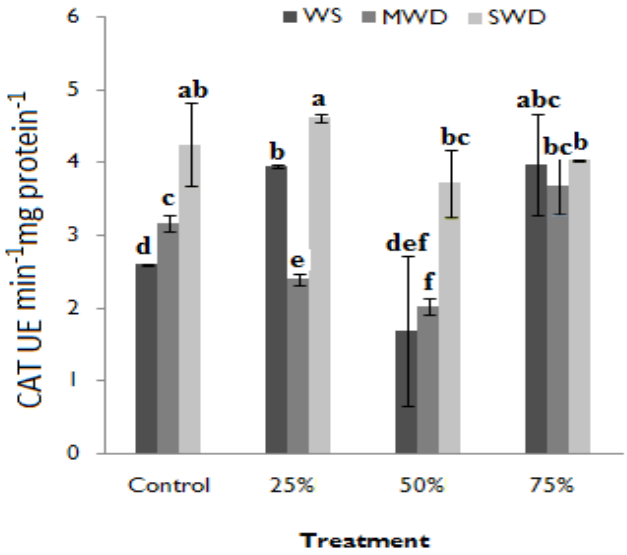


**Figure 6** The effect of water deficit on CAT activity in plant leaves of *Salvia officinalis* sprayed with sprayed with SWE. Results are means±SD (n=3).Different letters for each mean show statistically significant differences at P<0.05

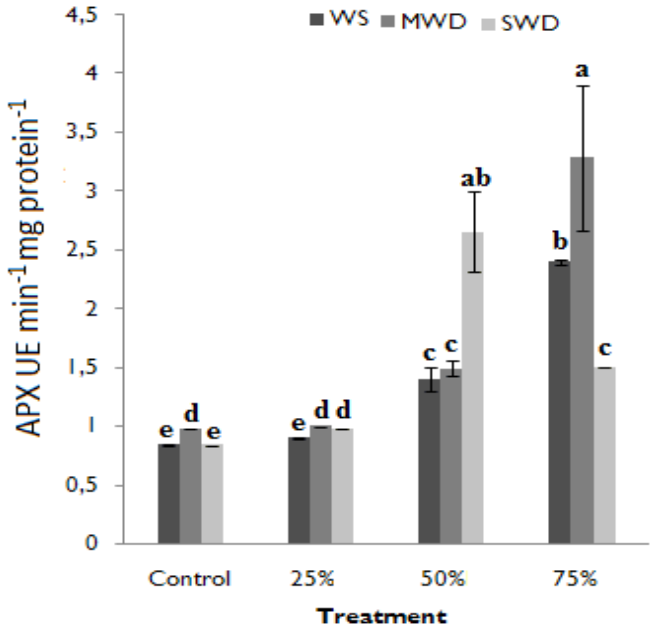


**Figure 7** The effect of water deficit on APX activity (C) in plant leaves of *Salvia officinalis* sprayed with sprayed with SWE. Results are means±SD (n=3).Different letters for each mean show statistically significant differences at P<0.05

CAT and APX were other important antioxidant enzymes that convert "$H_2O_2$" to water and were indispensable for ROS detoxification during stressed conditions [47]. The results demonstrated that CAT activity enhanced after water deficit, however APX activity exhibited no significant difference. Enzymes like APX which is located in peroxisomes and glyoxysomes [48]**.** Treatment by SWE enhance CAT and APX activity in our results, with this effect we can confirmed the important function of SWE extract in the tolerance to water deficit [49]. Ayad [50] reported that the activity of antioxidant enzyme like APX, CAT improved after treatment by seaweed extract. The strong antioxidant properties of seaweeds which have been correlated to bioactive compounds [51].

## Conclusion

In conclusion, plants of *Salvia officinalis* support the drought stress after treatment by seaweed liquid extracts of *Fucus spiralis* by reducing the effect of water stress with an enhancement in the morphological parameters, enzymatic and non enzymatic antioxidant parameters of plants. The application of SWE improve plants height, leaf area, and number of leaves in some Fucus treatment. Also we noticed the improvement of antioxidatifs system activity by activation of enzymes (APX, SOD and CAT) and increasing total phenolic with the diminution of MDA which indicate the protection of plants against lipid peroxidation imposed by water stress. However, the seaweed components of Fucus like cytokinine, betaine and oligosaccharides plays important role in this tolerance with synergistic activity.

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