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The use of seaweed as a bio-fertilizer: Does it influence Proline and Chlorophyll concentration in plants treated?

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Abstract: The objective of this study is to evaluate the influence of marine algae as biofertilizer on growth parameters and on the accumulation of chlorophyll "**a**" and proline in sunflower plants. Sunflower seeds are grown in medium containing green algae: *Ulva Lactuca* as biofertilizer and in two control media containing soil and soil with chemical fertilizer. After the 47th day of the growth, the highest growth parameters are found in the plants grown in the medium with seaweed. The highest rate of chlorophyll "**a**" was found in the leaves of these plants, while the roots of these plants have accumulated the lowest rate of proline. These results suggest that plants grown in media with seaweed were confronted with a low stress compared with those grown under control, which results in a better growth. Seaweed contributes to the best development of plants and also reduces the risk of biotic and abiotic stress and can be used as alternative to chemical fertilizers known to be harmful for health and the environment.

Keywords: Chlorophyta, *Ulva lactuca*, Bio-fertilizer, Growth Parameters, Chlorophyll, Proline.

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Introduction:

The advantages of seaweed as a source of organic matter and nutrients led to their use as a natural fertilizer soil for centuries. The benefits of applying seaweed farming have been highlighted by several studies: enhancement of seed germination and yields (Crouch et al. 1994; Arthur et al. 2003; Masny et al. 2004; Norrie and Keathley, 2006; Miyashita et al. 2013; Ganapathy et al. 2014; Shanga et al. 2014, Chenping et al. 2015), resistance to stress and increase chlorophyll content (Blunden et al. 1997; Thitumaran et al. 2009), decreased the incidence of insects and fungal pathogens (Washington et al. 1999). Numerous studies have also demonstrated a broad spectrum of beneficial effects of extract of algae applications on plants, such as a high resistance to biotic and better preservation during post-harvesting of perishable goods (Zhang et al. 2003). Chlorophyll is responsible for the green color of plant's pigments. This pigment, which is found in plant cells, is used with other pigments in plants to perform photosynthesis. This process allows the plant to use the sun's energy to convert carbon dioxide (CO_2) and water into oxygen and organic matter. There are several photosynthetic pigments (chlorophyll "**a**", "**b**", "**c**", carotene, phycocyanin, xanthophyll), but the most common is the pigment chlorophyll "**a**". It is found in all plants, algae and cyanobacteria (Chengkui et al. 1980). In plants, betaines are a compatible solute that reduces the osmotic stress induced by salinity and drought. Other roles have also been suggested, such as improving the chlorophyll content of leaves of plants after treatment with algae extracts (Blunden et al. 1997). This increase in chlorophyll content can be due to decreased chlorophyll degradation (Whapham et al. 1993). The accumulation of proline, induced by stress, can be the result of three complementary processes: stimulation of its synthesis, oxidation inhibition and / or alteration of the protein biosynthesis. Proline is synthesized from the glutamic acid via pyrroline 5-carboxylate (P5C) but also via the arginine and ornithine (Tahri et al. 1998). The accumulation of proline has been demonstrated in many species in different situations of stress (osmotic, water, heat) (Hossain et al. 2014). Some authors believe that the accumulated amounts could be related to the level of stress tolerance. Proline accumulation could play a role as osmoticum. It could also be involved in the regulation of cytoplasmic pH or constitute a reserve of nitrogen used by the plant after the period of stress (Tahri et al. 1998). Plants accumulate a number of osmoticums such as proline, betaine and carbohydrates (Wang et al. 2003). The accumulation of proline was demonstrated in many

species in different stress situations (osmotic, water and heat) (Hare et al. 1999). Proline protects the structure of the folded proteins against denaturation, stabilizes cell membranes by interacting with phospholipids. It is also responsible for other functions adapted to environmental stress conditions (Jiménez-Bremont et al. 2006; Tripathi et al. 2007). Plants treated with seaweed liquid extract exhibit tolerance to salinity and low temperatures (Mancuso et al. 2006; Erulan et al. 2009) indicated that the seaweed as fertilizer at low concentrations enhance the biochemical parameters such as chlorophyll content 'a' and 'b', sugar, proteins, as well as starch.

The aim of this study is to determine the amount of chlorophyll "**a**" in the leaves and proline in the roots of plants that seedling in a support with seaweed as bio-fertilizers and compare them with plants that seedling in only soil and soil with chemical fertilizer as control.

Materials and methods:

Collection of seaweed: Bio-fertilizer agent

The Chlorophytae green algae; *Ulva lactuca* was freshly collected manually from the coastal area of the Mediterranean, El Mina (34° 26'N-35° 50'E) in Tripoli, Lebanon during the month of March 2014. It was washed with seawater to remove all the unwanted impurities such as adhering sand particles and epiphytes. Then, the seaweed was transported in polyethylene bags which were moistened in the laboratory and they were dried at room temperature away from light.

Growth promoting efficiency of seaweed as bio-fertilizer on sunflower seedlings

The support was composed of green algae *Ulva lactuca* (5%) as bio-fertilizer, *Luffa aegyptica* (3%) as water retender and *Agar-agar* (92%) as plasticizer (Chbani et al. 2013). Two supports are used as control: one containing the soil and the other containing soil with chemical fertilizer.

The six white sunflower seeds, type of Vilmorin, with a uniform shape, size, color and weight were incubated in water at 30°C for 48 h before inoculation. Both of treated sunflower seeds have been sown at a depth of 1 cm in each support. Then the three supports were placed in a mini greenhouse at 22 to 28°C, 70 to 85% relative humidity, 600 to 1000 µmol photons m^{-2} s^{-1} light intensity and a photoperiod of 12 h during the observation period. The seeds were

watered regularly. The root system, the length and diameter of the stem and leaf area was measured a 2-days interval within 47 days. All samples were realized in triplicate.

***Dosage of chlorophyll* "*a*".**

The extraction was performed by the method established by (Holden 1965). So, 1 g of leaf pieces ground in a mortar with 20 ml of acetone 80% and a pinch of calcium carbonate ($CaCO_3$). The solution is then filtered and stored in the dark (in black boxes) to prevent oxidation phenomenon of chlorophyll due to light. The absorbance was determinate by UV spectrophotometer at 645 and 663 nm wave's lengths. The acetone solution at 80% was used as control.

The values of chlorophyll "**a**" are calculated according to the following formula (Arnon 1949): **Chl. a** = 12.7 (A_{663}) - 2.69 (A_{645})

Where A_{663} was the absorbance at 663nm wave's lengths and the A_{645} was the absorbance at 645nm wave's lengths.

Dosage of proline

Proline was assayed by the method of Troll and Lindsley (1955), as amended by Monneveux and Nemmar (1986). 100 mg of chopped fresh roots were added at 2 ml of 40% methanol. The mixture is heated in a water bath at 85°C for 60 min. After cooling, 1 ml of the extract is withdrawn then added to one ml of acetic acid (CH_3COOH) and one ml of a mixture containing 12ml of distilled water, 30ml of acetic acid, 80 ml of orthophosphoric acid (H_3PO_4 density 1.7) and 25 mg of ninhydrin. The solution is boiled for 30 minutes, it gradually turns red. After cooling, 5 ml of toluene are added to the solution and after stirring two phases are formed, the upper one which contains proline is recovered and dried by the addition anhydrous Na_2SO_4.

Finally, the absorbance was determined by a spectrophotometer at the wave length of 528nm. The control is a mixture of acetic acid, distilled water, orthophosphoric acid and ninhydrin.

Results and discussion

Results

***Effects of green algae; Ulva lactuca on the growth parameters of the sunflower plants*.**

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The evolution of growth on the 3rd to 98th day of sunflower plants in the support of *Ulva lactuca* green seaweed as bio-fertilizer showed higher parameters growth and healthy leaves as shown in **Figure 1**. On the 37th day of growth, sunflower plants grown in the soil began to turn yellow. On the 39th day those plants began to fade, and on the 47th day the plants died (Fig.2). Compared with the control medium (soil), our media-based green algae promote germination and similar and even higher growth with better value of growth parameters at 47th day: length of the stem **L** (61cm); stem diameter **D** (0.53cm) and leaf area **La** (67.27cm^2) (Tab1).

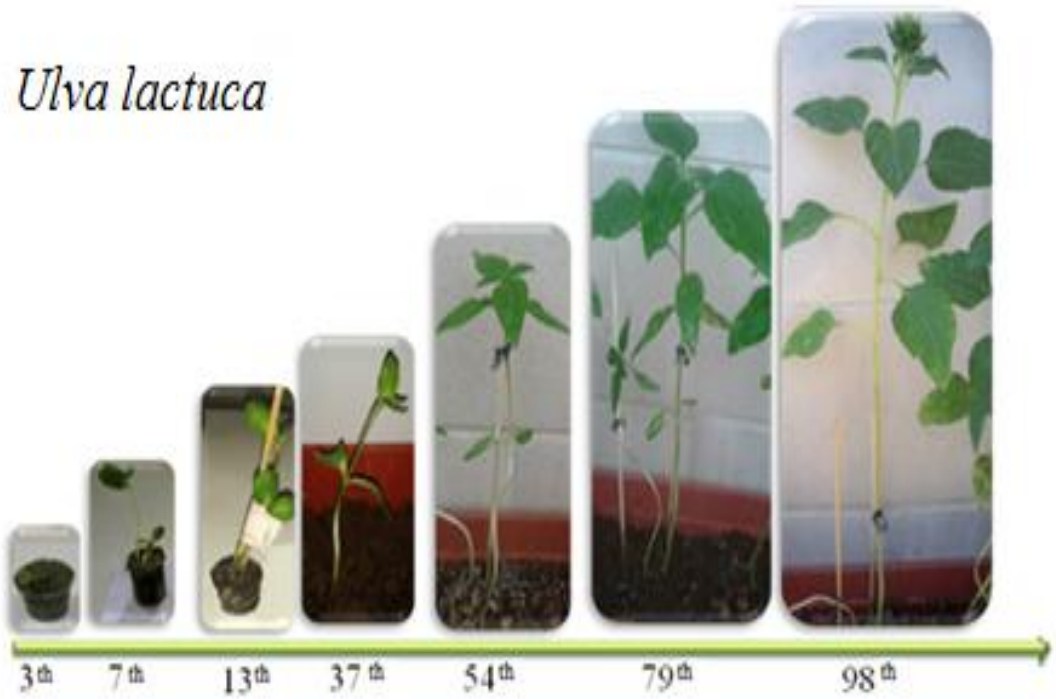

Figure 1. Evolution of the sunflower plants from the 3rd to the 98th day of growth in the support with green alga *Ulva lactuca* as biofertilizer.

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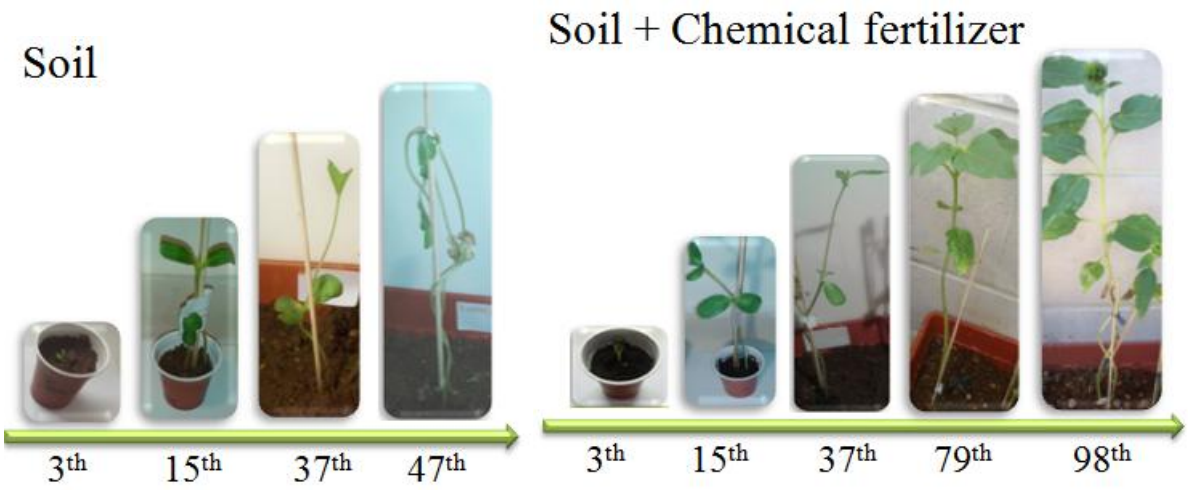

(a) **(b)**

Figure 2. Evolution of sunflower plants grown in soil from the 3rd to the 47th day **(a)** of growth and in the soil with chemical fertilizer from the 3rd to the 98th day of growth **(b)**.

Table 1: The growth parameters. L: length of the stem (cm); D: stem diameter (cm); La: Leaf area (cm^2) on the 47th day of growth.

Growth Parameters (47th day)	L (cm)	D (cm)	La (cm^2)
Bio-fertilizer: *Ulva lactuca*	61	0.53	67.27
Soil+ Chemical fertilizer	52.1	0.48	52.52
Soil	22.89	0.32	8.45

Concentration of chlorophyll "a" (mg/l)

The graph (fig. 3) shows the results of chlorophyll "**a**" leaves' concentration in the plants grown in different medium where the maximum concentration of chlorophyll "**a**" (247.9mg/L) was found in the leaves of plants that grew in the support base of seaweed *Ulva lactuca*.

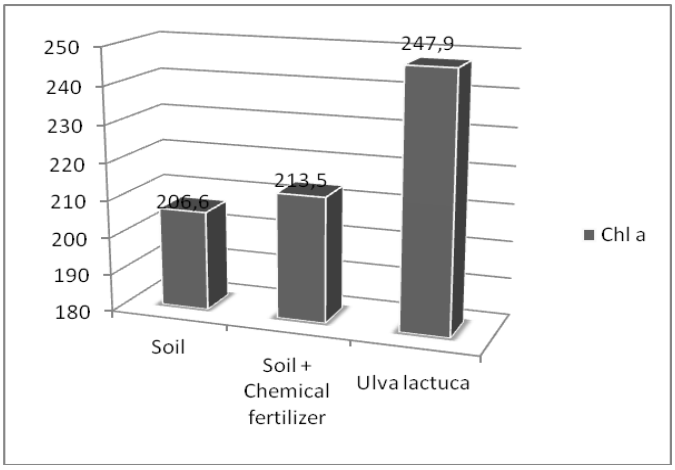

Figure 3. The concentration of chlorophyll "**a**" (mg/L) in the different media.

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Concentration of proline (mg/L)

The graph (fig. 4) shows the results of the proline dosages at the roots of the plants cultivated in various supports where the minimal concentration (0.4 mg/L) is found in the roots of plants that grew in the support based on algae.

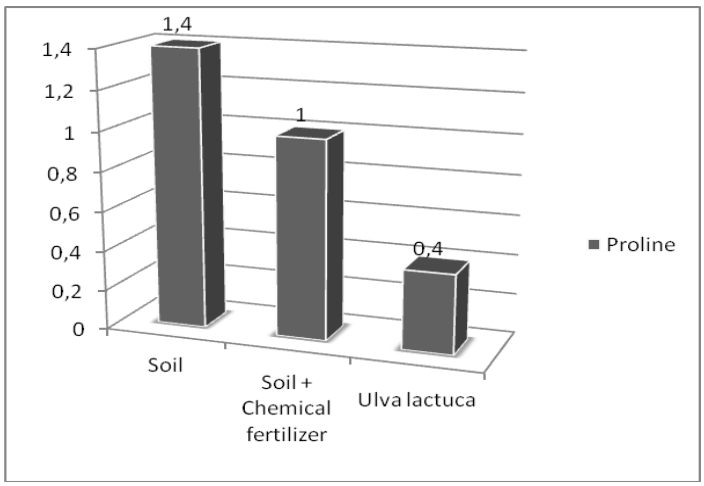

Figure 4. The concentration of the proline (mg/L) in the different media.

Discussion

The growth parameters (Stem length, stem diameter and leaf area on the 47th day of growth) and chlorophyll concentration in the leaves are higher in plant grown in support with seaweed *Ulva lactuca* as bio-fertilizer than the control. The proline concentration is lower in the root of this plant. Our results are in correlation with those of the literature. Indeed, treatment with the "seaweed liquid extract as fertilizer also increased the chlorophyll content" (Thirumaran et al. 2009; Ganapathy et al. 2014; Chenping et al. (2015). These findings coincide with some results as those obtained by (Whapham et al. 1993) who observed that the application of Ascophyllum nodosum seaweed liquid fertilizer increased chlorophyll content of cotyledons of cucumber and tomato plants. According to Matysiak el al. (2011) in the variant involving the seed soak and spraying plants with algal solutions, a significant chlorophyll concentration is obtained in comparison with the control. Ismail et al. (2011) demonstrate that the addition of seaweed as bio-fertilizers has increased the "growth parameters as well as fresh and dry matter of shoots and roots". Increased chlorophyll content of leaves was also observed in this study. These results are in agreement with those obtained by Khan et al. (2009) and Kumar

and Sahoo (2011). They found that treatment plants by seaweed liquid extract presented better quality of growth.

The algae can retain nutrients and release them slowly to the plants. It contains important nutrients such as nitrogen (N), phosphorus (P), potassium (K), calcium (Ca) and sulfate ($SO4^{2-}$) that will become available to the plants after decomposition (Khan et al. 2009). The decomposition of the algae is taken in charge by micro-organisms that degrade the algae into carbon dioxide, water and nutritious substances for the algae (mineralization). Seaweed also tends to enrich the medium with hormones such as gibberellins (Wildgoose et al. 1978), betaines (Whapham et al. 1993), cytokinins (Durand et al. 2003) and auxins (Stirk et al. 2004). They contain in addition, precursors of compounds elicitors that promote germination, growth and maintenance of the health of the plant (Ismail and Kardoush 2011). Our results correlate with those in the literature.

Assay results of proline showed that the roots of sunflower plants grown in media with seaweed have the lowest content of proline. In many plants, free proline accumulates in response to the imposition of a broad biotic and abiotic stress (Tahri et al. 1998). These results suggest that the plants grown in media with seaweed were confronted with a low stress compared with those grown in the control, which results in a longer growth. The accumulation of proline is a common metabolic response of higher plants to water deficit and salinity stress, and has been the subject of much criticism over the past 20 years (Samaras et al. 1995; Taylor 1996, Rhodes et al. 1999). This accumulation can involve in part the "induction and/or activation of enzymes proline biosynthesis". In addition, abiotic stresses such as drought, salinity and extreme temperatures can reduce the yield of most crops (Wang et al. 2003) and limit agricultural production worldwide. Taken together, the studies suggest that seaweed products raise the abiotic stress tolerance in plants and bioactive substances derived from algae which confer stress tolerance and improve the performance of plants.

Some proportionalities, but conversely, between the levels of accumulated proline and chlorophyll pigment concentrations are noticed. The variety that accumulates more proline is also the one that is the fastest to decrease in levels of chlorophyll pigments and vice versa. These results suggest the existence of a connection between the likely pathways of biosynthesis of chlorophyll pigments and proline. Competition between these two compounds on their common precursor, glutamate may be the cause of this trend (Tahri et al. 1998). The

decrease in chlorophyll may be due to the formation of proteolytic enzymes such as chlorophyllase, which is responsible for the degradation of chlorophyll that can damage the photosynthetic apparatus (Levent Tun et al. 2008). Our results agree with these studies; in fact, plants grown in the support base of seaweed stimulate plant growth, showed the highest chlorophyll levels and the lowest rate of proline.

Conclusion:

The high rate of chlorophyll in the leaves of plants grown in a medium based alga growth improves the quality of these plants. The low concentration of proline in the roots of these plants makes them a better resistant to biotic and abiotic stress. These results suggest the use of seaweeds as bio-fertilizers to promote growth and decrease stress of plant.

Competing interests

The authors declare that they have no competing interests.

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