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Impact of Water Stress and Marine Algae Spraying on Vegetative and Biochemical Traits of Hibiscus tiliaceus

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ABSTRACT

The experiment was conducted in the lath house covered by saran belonging to the preparatory school of agriculture in Abu Gharaq for the spring season 2022/2023. The Hibiscus tiliaceus plant is considered the latest type of tree to have spread recently in Iraq, due to its ability to withstand harsh environmental conditions and the beauty of its large yellow flowers. Water scarcity in Iraq is considered one of the biggest pressures and problems facing plant growth, especially in the early stages of agriculture. Therefore, the current study was conducted on the effect of water stress and spraying with marine algae on the vegetative traits and active compounds of the Hibiscus tiliaceus plant, where three traits were studied. The first trait is the area of the plant's leaves. The highest rate of increase was obtained in leaves of (159.107 cm^2), with exposed to spraying with seaweed at a rate of 4 ml/L, the second characteristic is the increase in the diameter of the branches, where the highest rate of increase reached (16.232 mm) when water stress was applied for 10 days. The third characteristic was the percentage of chlorophyll in the plant, where the highest rate of chlorophyll reached (36.883 CCI) when a water stress of (10 days) was applied.

1. INTRODUCTION

The Hibiscus tiliaceus plant is considered the latest type of tree that has spread in Iraq recently due to its ability to withstand harsh environmental conditions and the beauty of its large yellow flowers. Water scarcity in Iraq is considered one of the biggest pressures and problems facing plant growth, especially in the early stages of agriculture [1].

The method of treating plants with seaweed is one of the methods used recently in the agricultural field. It is a natural material that does not contain any chemical substances in its manufacture, which would increase pollution in the environment [2].

Algae are multicellular photosynthetic organisms that do not have true roots, stems, or leaves. They live in brackish ocean waters as well as in fresh and very cold waters. In return, these algae make their living conditions much more favorable by means of their mineral content. All algae vary in size, some have cell sizes so small that they are only visible, under a normal microscope while others can be so large, that can be seen by naked eyes [3].

The environmental circumstances found in ecosystems, among others, include saline media exposure, strong winds and water scarcity, which pose numerous challenges to the growth and resilience of vegetation that thrives in such regions [4]. Hibiscus with the commonly known as beach hibiscus has soft flowers and large leaves. One interesting thing is its ability to cope with stressful water conditions as it occurs in arid or semi-arid regions and that is the reason seaweed irrigation should be put under the microscope to reveal its influence on ecosystems in general and plant diversity and productivity in particular [5].

Coastal areas often face water shortages due to negative factors, like water salinization, bad water quality and high humidity. Water stress generally acts as a trigger for plants to develop adaptations like reduced leaf size and change in the root features or in growth patterms. Its impacts could range from simple changes in the ecosystem to more complex ones [6].

Seaweeds especially with their range variety have been found to emit an enormous amount of biomass; indeed, numerous studies have reported about the near future benefits of the seaweed for increasing crop yields and resistance together to stress [7]. The application of seaweed sprays or solutions to plants has been studied by researchers as a potential method to enhance plant performance and possibly mitigate negative environmental effects [8]. Having the purpose to look into the impacts of seaweed spraying on marine vegetation, the researchers will reveal the underlying metabolic changes in the main target plants in 1999, and their activities, including the changes occurring in the chemicals themselves, which may influence the ecological contacts of such plant with other species and thus their suitability for the use in various manufacturing processes [9].

The goals of the study were to disclose and categorize the effects of drought stress and a change in seaweed management of Hibiscus tiliaceus [10]. Through the study of this coastal plant's water stress and seaweed response, researchers will benefit from the detailed finding thereby providing information on the plant's adaptability mechanisms, possibilities of stress downstream, and what changes in the secondary metabolites [11]. The results of the study can add to the knowledge on how plant proliferate in marine environments and provide useful data to the sea coast safeguarding and sustainable use. Possibilities of the research findings being utilized in fields as diverse as agriculture, medicine, and even other disciplines are enormous [12].

2. LITERATURE REVIEW

Our research demonstrated that seafood extracts in high concentrations stimulated, fruiting and roots, biochemical elements, which includes lycopene and vitamin C. Seaweed extracts showed activity which is related to the auxin and cytokinin, as well as contained useful macro- and micro-nutrients proved them an efficient biofertilizer [13].

The biofertilizer potential of the brown algae Sargassum johnstonii for tomato plants was investigated. Different concentrations of seaweed extracts were applied by foliar spray, soil irrigation, and a combination of both methods. Tomato growth, yield and biochemical composition were monitored over a period of seven months [14].

The study examined the effects of water extraction of various red, brown and green algae on root length of wheat stalks. Low and very low concentrations of seaweed extract have been found to stimulate root elongation [15]. Among the algae tested, Neorhodomela larix, Tichocarpus crinitus, Saccharina japonica, and Codium fragile showed the highest potency as biostimulants, increasing root length by up to 20% compared to the control. The dose-effect curves of the seaweed extracts varied, with some exhibiting bimodal or polymodal responses [16].

This study focuses on the extraction, separation, estimation, and isolation of chlorophylls. Chlorophylls are the primary green pigments found in autotrophic and photoheterotrophic organisms, extracted using organic solvents [17]. The chapter discusses the various alteration products that arise from natural green pigments due to injury, reagents, or unfavorable conditions. Chromatographic methods, including columnar, paper, radial, and thin-layer chromatography, are used for qualitative and quantitative analysis of chlorophylls [18]. These methods enable identification, sequencing, and estimation of individual pigments through fluorimetric, calorimetric, or spectrophotometric techniques [19].

A water stress response undoubtedly involves the activation of complex biochemical pathways. Taking advantage of the basic knowledge already acquired from other model species, it is reasonable to include analyses of behaviors of some secondary metabolites, such as phenols, and the antioxidant activities of the leaves. Although there have been discrepancies regarding the parts and species of algae used, on average, algae treatments have improved vegetative traits, photosynthetic pigments, and antioxidant enzymatic activities in plants responding to water deficits. International policy development has mostly been focused on increasing productivity and sustainability. One way of working on this issue is to apply genetic improvements to commercial varieties. Other strategies for studying the development of innovative commercial varieties resilient to environmental stresses have been introduced. The need for improvement has been validated by increasing research into the potential use of bio-elicitors, such as marine algae extracts, which promote plant growth by providing a supportive framework to stressed crops.

3. MATERIALS AND METHODS

The experiment was conducted in the lath house covered by saran belonging to preparatory school of agriculture in Abu Gharaq for the spring season 2022/2023. To study the effect of water stress and spraying with marine algae on the vegetative traits and active compounds on Hibiscus tiliaceus trees planted at a distance of (3 × 4 m), based on completely randomized block design (B.C.R.D) with three replicates and each tree representing one experimental unit.

Selection of 45 trees that were as homogeneous as possible in terms of the size of the shoots was done through this. The paper is a study of two factors, the first factor is the water stress and its effect on the growth and development of the Hibiscus tiliaceus plant and physiological processes, including the area of the plant leaf, the increase in the diameter of the branches, and the total chlorophyll in the leaves, which leads to changes in plant characteristics and active compounds [20]. The second factor is spraying coastal Hibiscus tiliaceus plants with seaweed. Seaweed, also known as seaweed, is used as a therapeutic method in this study. Spraying seaweed extracts or solutions on plants is being explored as a potential approach to enhance plant performance and mitigate the negative effects of water stress. Application of seaweed has effects Specific biological, physiological and biochemical effects on the plant, affecting its vegetative traits and increasing the production of active compounds.

The design of the study was a completely randomized design with three kinds of treatments and seven replicates per treatment. Before the start of the experiment, suitable plants were chosen after recording some initial vegetative and physiological traits. In order to have before and after measurements, all types of treatments were carried out in parallel. The groups that involve only the spraying of marine algae are not included in the design and do not run in parallel with the treatments, as this would not allow for the evaluation of the efficiency of marine algae under different water regimes [21]. This is fundamental for understanding the real feasibility of applying this innovative approach, especially when treatments start from seedlings, a very useful approach in the context of seawater agriculture to obtain a resistance trait from the seedling phase. Three groups were arranged: control (T0—1 daily irrigation per day), light water stress in terms of VOC decrease (T1—70% daily reposition), light-moderate water stress in terms of Fv/Fm decrease (T2—40% daily reposition), in compliance with the thresholds identified. Thus, these thresholds were suitable for inducing different levels of stress in the plants, negativity, and decline in the measured parameters, affecting their growth, as shown in the results.

The duration of the treatments was adequately decided, reasoning that the intervals between the repositioning of daily irrigations, except for T0, had to allow a progressive increase of water stress in the plants that, theoretically, would have reached very high values. The experiment was carried out in the experimental greenhouse of the Department of Agricultural Sciences, located in Portici. The treatments were performed while maintaining both agronomic and environmental conditions suitable for plant growth. The allocation of the treatments started in the evening and was carried out in such a way as to balance the possible bias due to the use of fertilizers and plants. After ten days from classifying the plants, the treatments were arranged and monitored every 7 days. The data collected served for statistical analysis to understand which treatment was most effective. The water flow index was analyzed using an analysis of variance.

3.1 Hibiscus tiliaceus

It is a relatively small tree with a height of about 10 meters. Its native habitat is Southeast Asia, the Pacific Islands, and, in general, the southern coastal areas below the equator. This genus includes many species exceeding 200 species. Its flowers are abundant in the spring, and appear intermittently throughout the year. The lifespan of the flower often does not exceed one or two days, after which it turns orange. The flowers consist of light yellow petals. The flower is marsupial-shaped, 15 cm wide, and the carpel is long, protruding from the center of the flower. The color is crimson, as shown in Figure 1. The flowers fall one or two days after they open.

Figure 1. Hibiscus tiliaceus tree with large yellow flowers

3.2 Seaweed

Seaweed, also known as marine algae, is a diverse group of photosynthetic organisms that inhabit marine environments. They are classified as macroalgae and can be found in coastal areas, oceans, and even freshwater bodies. Seaweeds come in various shapes, sizes, and colors, ranging from microscopic unicellular forms to large, multicellular species that can reach several meters in length. Seaweeds play crucial roles in marine ecosystems and have significant ecological importance. In the spray application for this study, the seaweed extract was diluted to the required concentration using an appropriate diluent (distilled water). The concentrations of algae used in this study are (1.5, 4) ml^{-1} as shown in Figure 2, the diluted extract is then applied to the Hibiscus tiliaceus plant using a hand sprayer or specialized spray device. Care is taken to ensure comprehensive and uniform coverage of plant surfaces with the extract solution. In control groups Control groups are created where seaweed plants are treated with diluted water without seaweed extract. These control groups allow comparison of effects between treated and untreated plants.

Figure 2. Seaweed spraying system

During the trial period, the plants are monitored and cared for according to their specific requirements, including appropriate lighting, temperature and irrigation systems. The duration of the experiment may vary depending on the objectives of the study, but in this study it extended to 3 months.

A reliable and repeatable protocol is indispensable for comparison between the present data and those of similar investigations. New research systems should always be tested on one species in several laboratories before being widely accepted. The materials and methodology sections provide a clear and concise description of the test materials, how they were obtained, and the methods used. Two different types of marine algae were harvested [22]. Both of them were processed and received at the experimental station. Then diluted to a 2% stock preparation in water for tape. Plant treatment by spraying a solution rich in minerals and vitamins was dissolved in water for tape; after two simultaneous plant treatments, the pots were placed in the controlled environment cabinets. The growth of the plant was monitored, leveling the enviable condition until 93 days after the plants' sowing. The upper part of the plants was cut for chlorophyll analysis. Data have been analyzed using the F test. The critical level of significance was set at 5% or 1% using statistical software. The least significant difference test was used for calculation. The physiological data were subjected to one-way analysis of variance, and the means were tested by the least significant difference test. A correlation analysis was performed between the measured physiological parameters.

3.3 Characteristics studied

Plant leaf area cm^2, it was calculated using the (Digimizer software), as the papers were scanned using an Hp scanner, and it was done, and placed a white board behind the papers and drew on the board a colored line 10 cm long next to the papers and transferred the picture to the software calculated the paper area and took measurements in the month of May. Increase in branch diameter (mm), the measurement of the vegetative branches was taken using the (Vennier) measuring tool, from the base of the branches at the beginning and end of the experiment, by choosing four branches in different directions for each experimental unit, and the difference between the two readings represent the increase in branch diameter. Total chlorophyll in leaves, estimate the chlorophyll content of leaves in the field using Content Chlorophyll (CCM200) meter plus. The physical and chemical characteristics of the soil of the research field were arranged as in Table 1.

Table 1. Physical and chemical characteristics for soil of the study

Characteristic	Measuring Unit	Value
Electrical conductivity	Ds. m^{-1}	0.94
Electrical conductivity For irrigation water	Ds. m^{-1}	2.8
Degree of soil reaction (pH)	-	7.5
Organic matter	%	1.36
Total nitrogen	Mg.K^{-1}	8.9
Total calcium	Mg.K^{-1}	1.7
Sand	g.Kg^{-1}	425
Silt	g.Kg^{-1}	326
Clay	g.Kg^{-1}	254

Nine seedlings nurtured in a polybag were treated per treatment—namely water stress and seaweed. The seaweed of Gracilaria spp. was manufactured by a small-scale industry producing seaweed chips. We believe that the population of these algae is placed in a quite clean site. The stem/leaf biomass (50:50) was used, whereas the water extract was prepared at a range of 5000, 10000, and 20000 ppm, produced by soft and hard methods. Thus, there are two independent variables, i.e., the concentration and time of spray on the bio-stimulant [23]. The water stress treatment was merely used as a control plant. Therefore, distilled water of equal volume to the chemical applications was applied to the stressed trees and the control plant.

Measurement of morphological parameters: Hibiscus tiliaceus shoots after the first stage (30 days old) and the second stage (60 days old) were collected to measure shoot length (cm) and fresh plant weight. The collected samples were subjected to oven drying at a temperature of 70℃ until the weights were constant it was relied upon to measure the dry weight of the Hibiscus tiliaceus plant and the percentage of water content in the plant buds estimated according to the following equation:

$$Water\ content(\%) = \frac{Mf - Md}{Mf} \times 100 \quad (1)$$

where, Mf is fresh mass, Md is dry mass.

Concentration the percentage of chlorophyll in the tissues of Hibiscus tiliaceus was calculated with the following equations:

$$Chl - \frac{a}{g} plant\ tissue\ = 11.63(OD\ at\ 665) - 2.39\ (OD\ at\ 649) \quad (2)$$

$$Chl - \frac{b}{g} plant\ tissue\ = 20.11(OD\ at\ 649) - 5.18\ (OD\ at\ 665) \quad (3)$$

Total chlorophyll:

$$\frac{a\ +\ b}{g} plant\ tissue\ = 6.45(OD\ at\ 665) + 17.72(OD\ at\ 649) \quad (4)$$

4. RESULTS AND DISCUSSION

It is clear from the results shown in Table 2 that there is a significant effect of spraying with marine algae, as spraying with algae at a concentration of 4 ml^{-1} caused a significant increase in the area of one leaf, 159.107 cm^2, as it was significantly superior to all the algae treatments used. However, the lowest rate for this characteristic was 124.249 cm^2 when spraying with algae was missing, Algae contains many nutrients, such as nitrogen, phosphorus, and potassium, which when sprayed on plants act as a natural fertilizer. These nutrients improve plant growth and essentially increase the size of its leaves, which leads to healthier, more productive plants.

Table 2. Effect of water stress and spraying with seaweed on the area of one leaf (cm^2) trees

Seaweed / Water Stress	S_1 0 ml^{-1}	S_2 1.5 ml^{-1}	S_3 4 ml^{-1}	Rate of Seaweed Effect
H_1, 3 days	116.463	130.497	134.407	127.123
H_2, 8 days	125.737	155.943	164.387	148.689
H_3, 10 days	130.547	139.377	178.527	149.483
Rate of water stress effect	124.249	141.939	159.107	

Turning to the water stress data in the same table, it becomes clear to us that there are significant differences between the stress values in this characteristic, the stress of 10 days was significantly superior in giving a higher rate of the leaf size trait, reaching 149.483 cm^2 when compared to the lowest rate of the studied trait, which reached 127.123 cm^2 at the highest water stress used for the study, which was 3 days, the reason for this can be explained as reducing water stress leads to increased water supply, as plants can produce larger and more abundant leaves, which contributes to increased leaf area indicators of the plant, and thus leads to improved light interception and plant productivity in all forms.

Table 3. Effect of water stress and spraying with seaweed on the increase in the diameter of the branches (mm)

Seaweed / Water Stress	S_1 0 ml^{-1}	S_2 1.5 ml^{-1}	S_3 4 ml^{-1}	Rate of Seaweed Effect
H_1, 3 days	7.40	11.013	12.589	10.334
H_2, 8 days	10.32	15.211	15.356	13.629
H_3, 10 days	11.45	18.553	18.693	16.232
Rate of water stress effect	9.7233	14.926	15.546	

It is clear from the results shown in Table 3 that there is a significant effect of spraying with marine algae on the increase in the diameter of the branches. Spraying with algae at a concentration of 4 ml^{-1} led to a significant increase in the diameter of the branches by 15.546 mm. It was also significantly superior to all algae parameters used. However, the lowest rate for this characteristic was 9.7233 mm when spraying with algae was missing, the reason for this can be explained by the fact that algae possess growth stimulants, as algae produce growth-promoting substances, the most important of which are auxins and cytokinins, these plant hormones can stimulate cell division and overall growth, which enhances root growth, increases the growth of buds and branches, and improves the general activity of the plant.

Referring to the water stress data in the same table, we see that there are significant differences between the stress values in this property. The stress of 10 days was significantly superior in giving a higher rate of increase in the diameter of the branches, as it reached 16.232 mm when compared to the lowest rate for the studied characteristic, which amounted to 10.334 mm at the highest water pressure used for the study, which was 3 days. The reason for this can be explained reducing water stress enhances cell division and elongation, as providing adequate water is essential for cell division, which are important processes that contribute to the growth of plant tissues, including branches. Therefore, when water stress is reduced, the plant works to allocate more resources to new growth processes, which leads to increase cell division and elongation of the cambium and other meristematic tissues responsible for plant growth in general and branches in particular.

Table 4. Effect of water stress and spraying with seaweed on the increase in the total chlorophyll (CCI) of trees

Seaweed / Water Stress	S_1 0 ml^{-1}	S_2 1.5 ml^{-1}	S_3 4 ml^{-1}	Rate of Seaweed Effect
H_1, 3 days	23.211	28.456	33.564	28.410
H_2, 8 days	32.523	24.897	36.321	31.247
H_3, 10 days	33.655	36.342	40.653	36.883
Rate of water stress effect	29.7963	29.899	36.846	

It is clear from the results shown in Table 4 that there is a significant effect of spraying with marine algae in increasing the total chlorophyll of the plant. Spraying with algae at a concentration of 4 ml^{-1} led to a significant increase in chlorophyll by 36.846 CCI. It was also significantly superior to all algal standards used. However, the lowest rate for this characteristic was 29.7963 CCI in the absence of algae spraying, and the reason for this can be explained by the fact that Algae possess photosynthesis enhancers. They are living organisms that can carry out the process of photosynthesis efficiently when sprayed on plants. They work to transfer some photosynthetic pigments, such as chlorophyll, to the plant leaves and thus increase the overall photosynthetic capacity of the plant. This means an increase in the production of carbohydrates, and improved use energy to produce chlorophyll. Water stress in the same table contains significant differences between the stress values in this property, where the pressure of10 day was significantly superior in giving a greater rate of increase in chlorophyll production, reaching 36.883 CCI compared to the lowest rate for the studied property, which reached 28.410 CCI at the highest water pressure used for the study, which was 3 days, the reason for this can be explained by chlorophyll are molecules that belong to protein complexes known as photosystems, which participate in the process of photosynthesis. Water stress can disrupt protein synthesis, by reducing water stress, plants can restore protein synthesis, this means ensuring a significant increase in proteins and thus increasing chlorophyll production.

Free radicals are formed as a result of oxidation reactions in the body, and they are considered unstable compounds capable of causing damage to cells and tissues by interacting with biomolecules such as proteins, lipids, and DNA. Medicinal antioxidants play an important role in dealing with hydroxyl radicals and combating the harmful effects of oxidation in the body. Antioxidants work to neutralize free radicals and provide electrons to them, thus reducing their ability to harm cells. Antioxidants protect the body from the harmful effects of oxidation and prevent the formation of free radicals or reduce their concentration. Free radicals and excessive oxidation are considered one of the factors contributing to cell aging and the development of chronic diseases such as cancer, cardiovascular disease, and neurological diseases. Figure 3 shows laboratory scavenging rate of hydroxyl radicals by Hibiscus tiliaceus plant extract.

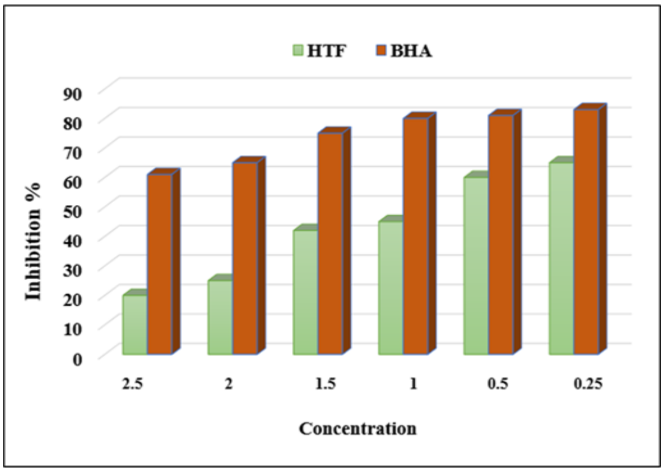

Figure 3. Inhibition of HTF and BHA (µg/ml)

5. CONCLUSIONS

The Hibiscus tiliaceus plant has emerged as a resilient tree species in Iraq, with its ability to thrive in harsh environmental conditions and its captivating yellow flowers. Limiting water supply becomes a serious issue for plants development during initial points in agriculture. This study was conducted in order to identify the changes in the vegetation geometry and the levels of active compounds after exposing Hibiscus tiliaceus to water deficit and supplementing it with the seaweeds. The research focused on three key traits: the leaves area, the stem diameter and the percentage of chlorophyll. Through data analysis, there were some interesting outcomes identified. Through marine algae spray, plant development underwent a significant increase in the length of their leaves when it was administered at a predefined concentration. Besides that, effectively exposing the plant to water stress resulted in considerable enlargement of branch diameter and augment of chlorophyll content. Thus it can be tell that seaweed spray and controlled water stress are useful in growing the Hibiscus tiliaceus and also leads at the improvement of its physiological characteristics. Specific habitat where this plant lives is the marine environment showing marked difference compared to absolutely freshwater. Immersion of knowledge in Hibiscus tiliaceus and other plants that are capable of growing in extreme environment leads to the investigation of various treatment processes including marine algae spray. This will provide the insights into their resilience and the applications. These makes the research practical since it allows better knowledge about plants reactions to the seaside situation. This information will be useful for the plant protection and the management of coastal ecosystems. Thus, the results are of future importance for agriculture and medicine based on the bioactive compounds of Hibiscus tiliaceus. This study underscores the beneficial effects of marine algae spraying on Hibiscus tiliaceus under water stress conditions. Immediate recommendations for growers include incorporating marine algae as a biostimulant to enhance plant resilience and productivity in drought-prone areas. The findings suggest broader applications in agriculture and potential benefits in pharmaceutical and environmental contexts.

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