Effect of foliar Spray With Ascophyllum nodosum Algae Extract and Tocopherol on Some chemical and vegetative Traits of Date Palm (*Phoenix dactylifera* L.) cv. Saqei under salinity stress conditions

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Abstract:

This experiment was conducted in private orchard located in Al-Hartha District (Al-Khait area), north of Basrah Governorate. A total of twenty-seven date palm trees (*Phoenix dactylifera L.*), cv. Saqei, were selected to evaluate the influence of two experimental factors. The first factor consisted of foliar application of Ascophyllum nodosum extract at three rates 0, 2.5, and 5 g L^{-1}, while the second factor involved foliar Spraying of tocopherol at rates of 0, 70, and 140 mg L^{-1}. In addition, the interaction between the two factors was also studied with respect to vegetative traits (number of leaves) and chemical traits of the leaves (total chlorophyll, carbohydrates, and proline).

The findings indicated that foliar treatment with Ascophyllum nodosum extract resulted in significant variations among treatments. The application Ascophyllum nodosum at rates of (5 g L^{-1}) recorded the highest values for the studied parameters (number of leaves, total chlorophyll, carbohydrates, and proline), reaching 5.89 leaves per plant, 3.46 mg per 100 g fresh weight, 21.06%, and 14.37 µg g^{-1}, respectively. As for the foliar Spray of tocopherol, the results revealed significant effect leaf number, total chlorophyll, and proline content, whereas no significant differences were observed in carbohydrate content. The concentration of 140 mg L^{-1} of tocopherol showed superiority by recording the highest mean values for the number of leaves and proline content, reaching 5.33 leaves per plant and 15.30 µg g^{-1}, respectively. In contrast, the concentration of 70 mg L^{-1} of tocopherol recorded the highest mean value for total chlorophyll, reaching 3.12 mg per 100 g fresh weight. The combined interaction of the two factors a significantly effected on the vegetative and chemical traits on leaf.

Keywords: Ascophyllum nodosum extract, tocopherol, Saqei cultivar, salinity stress.

Introduction

Date palm (*Phoenix dactylifera* L.) is one of the oldest cultivated fruit tree species known to humankind. Its widely grown in Iraq, the Middle East, and North Africa due to its high tolerance to arid and semi-arid environments. In addition, it is considered an important strategic crop because of its nutritional and economic value [1].

The Arab region is known forits rich diversity of date palm cultivars, with more than 3,000 varieties reported across different countries. Iraq is recognized as one of the main centers of this diversity, with over 600 identified cultivars [2]. the Sagei cultivar is commonly grown in Saudi Arabia, especially in the Riyadh area, where it ranks among the most abundant varieties. Its fruits are characterized by their elongated cylindrical shape and medium to

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large size, and they are consumed at both the rutab (soft ripe) and tamr (fully ripe) stages [3].

Salinity considered one of the major environmental stresses affecting agricultural production worldwide. High salt levels in soil or irrigation water can negatively influence plant growth and development by disturbing important physiological and biochemical processes such as photosynthetic, respiration rate, carbohydrate metabolism, lipid composition, and protein synthesis [4].

Seaweed extracts are widely used as natural plant growth promoters. They contain growth regulators, essential nutrients and antioxidant compounds that help reduce oxidative damage. In addition, they improve water and nutrients uptake, increase chlorophyll content and enhance plant tolerance to abiotic stresses such as salinity, drought and temperature fluctuations [5,6]. Extracts of Ascophyllum nodosum are among the most commonly used due to their positive effects on vegetative growth, including increasing leaf number and area, improving photosynthetic activity, and enhancing overall plant performance. They also help plant better tolerate unfavorable environmental conditions such as salinity, drought and low temperatures [7,8].

Antioxidants are substances that reduce or delay oxidative damagein plant cells by limiting oxidation reactions. They play a key role in controlling reactive oxygen species (ROS), which are highly reactive molecules capable of causing cellular damage. Antioxidants are broadly classified into enzymatic and non-enzymatic systems [9].

Tocopherols are an important group of non-enzymatic antioxidants that help protect plants from abiotic stresses such as salinity, drought, and low temperatures. They contributeare to maintaining membrane stability,especially in vascular tissues such as xylem and phloem, under stress conditions. In addition, tocopherols are effective inreducing the harmful effects of ROS and mitigating their cytotoxic effects [10,11,12,13].

Objective of the Study

This study was conducted to reduce the negative effects of salinity stress on the vegetative and biochemical traits of date palm by using seaweed extracts and antioxidant compounds.

Materials and Methods

Experimental Site:

The experiment was carried out in a private orchard Al-Hartha District (Al-Khait), north of Basrah Governorate. A total of 27 date palm trees (Sagei cultivar) were selected the trees were similar in size, height and growth condition, free from pests and diseases and aged between 4 and 5 years. The palms were planted at a spacing of 10 × 10 m in silty clay soil and irrigated (flood irrigation) from the Al-Mushab River. All trees were managed under the same agricultural practices. Each trees were marked and labeled according to treatment and replication.

Some physical and chemical properties of the soil and water samples:

A number of physical and chemical properties of the soil and water samples were analyzed and determined at the laboratories of the Date Palm Research Center, University of Basrah, as presented in Table (1):

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Table (1): Physical and Chemical Properties of the Soil and Irrigation Water at the Experimental Site

No.	Property	Values	Unit
1	(E.C)	23.84	$dS\ m^{-1}$
2	Soil pH	7.81	1:1
3	Organic Matter (O.M)	7.520	%
4	Soil Separates		
5	Sand	37	$g\ kg^{-1}$
6	Silt	322	$g\ kg^{-1}$
7	Clay	641	$g\ kg^{-1}$
8	Soil Texture	Silty Clay	
9	Properties of the Irrigation Water		
10	Electrical Conductivity (E.C)	24	$dS\ m^{-1}$
11	Determination of pH	7.39	1:1
12	Sodium (Na^{+})	29.26	$MEqL^{-1}$

Study Treatments, Their Concentrations, and the Timing and Method of Spray

The study included a factorial experiment consisting of two factors:

The first factor: Foliar Spray of the Ascophyllum nodosum Algae Extract at rates 0, 2.5, and 5 $g\ L^{-1}$.

The second factor: Foliar Spray of the antioxidant tocopherol at rates 0, 70, and 140 $mg\ L^{-1}$.

Spray solutions were prepared by dissolving 2.5 and 5 g, and 70 and 140 mg, respectively, per liter of distilled water. A surfactant (Tween 20) was added at a concentration of 0.1% to reduce surface tension, For the control treatment, distilled water containing Tween 20 only was used,Foliar spraying was applied to the vegetative parts of the plants in the early morning. The treatments were applied in three spray applications, with an interval of 30 days between each Spray.

Chemical composition of Ascophyllum nodosum extract:

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components of Ascophyllum nodosum extract include essential nutrients ,amino acids, vitamins,antioxidants, polysaccharides,andplant growth regulators such as auxins,cytokinins and gibberellins.

Studied Traits:

Vegetative Traits:

Upturn in Leaf Number (leaves. offshoot^{-1})

number of newly developed leaves was determined using the following formula:

Number of newly formed leaves = Total number of leaves after treatment − Total number of leaves before treatment

Chemical Traits:

Content total chlorophyll (mg .100 g^{-1}. fresh weight)

Content total chlorophyll in leaf samples was measured and estimated using fully expanded leaflets taken from the middle portion of the leaf, following the method described by [14].1 g sample of fresh leaf tissue was used, and total chlorophyll pigments were extracted using 80% acetone. The absorbance of chlorophyll pigments was measured at wavelengths of 663 and 645 nm using a spectrophotometer, Chlorophyll concentration was calculated according to the following equation:

Content total chlorophyll (mg L^{-1}) = 20.20 × O.D. (645) + 8.02 × O.D. (663)

Where Optical density (O.D) represents the absorbance reading,The results were then converted to mg 100 g^{-1} fresh weight using the following equation:

mg 100 g^{-1} = (mg L^{-1} / 1000) × (100 / sample weight)

Total Soluble Carbohydrates (%)

The total soluble carbohydrates present in leaf tissues were quantified following the phenol–sulfuric acid method according to [15].

Leaf samples were taken from (middle region) of the leaves for each treatment. thoroughly cleaned, and washed with running water. The samples were then oven-dried at 70°C until constant weight (after 48 hours),The dried samples were finely ground, and 0.5 g of the powdered dry sample was placed in a (100 ml) volumetric flask, followed addition of by the 70 mL distilled water. The mixture was sealed and incubated in a water bath at 90°C for one hour, after which it was allowed to cool to ambient temperature. Filtration was carried out using filter paper, 5 mL of the filtrate was diluted with 25 mL distilled water. Then, 1 mL of this solution was reacted with 1 mL of 5% phenol and 5 mL of concentrated sulfuric acid, then left to cool at room temperature.

Absorbance readings were recorded at 490 nm using a spectrophotometer. Total soluble carbohydrate content was determined using a standard glucose calibration curve, according to the following equation:

Total soluble carbohydrates (mg 100 g^{-1}) = (Carbohydrate concentration from standard curve × final extract volume × dilution factor) / sample weight

Determination of Proline Content (µg g^{-1})

Proline levels in leaf samples were determined based on the method described by [16]. A 0.2 g portion of dried and ground plant material was extracted using 5 mL of 95% ethanol. The extract was centrifuged, and the supernatant was collected and evaporated to complete dryness.The residue was re-dissolved in 2 mL of distilled water, centrifuged again, and the supernatant was collected. Absorbance was measured at 520 nm using a spectrophotometer.Proline content was determined using a standard calibration curve

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prepared with the amino acid proline. Results were expressed as µg g⁻ ¹ dry weight and calculated using the following formule:

Proline content (µg g⁻ ¹) = (value obtained from standard curve /sample weight) × dilution factor

Statistical Design and Applied Treatments:

The study was designed as a two-factor factorial experiment. The first factor involved foliar spray of the Ascophyllum nodosum algae extract at two rates. The second factor included foliar spray of the antioxidant tocopherol at two rates. All possible interactions between the two factors were examined, along with acntrol treatment.The experiment was arranged according to a Randomized Complete Block Design (RCBD) with three replicates. A total of 27 date palm trees cv. 'Saqi' were used in this study.

Data were statistically analyzed according to the applied experimental design using GenStat (2009) software. Treatment means were compared using the Least Significant Difference (LSD) test at a significance level of 0.05, following the method described by [17].

Vegetative Traits:

Increase in Leaf Number (leaves. offshoot⁻ ¹)

The findings presented in Table (1) demonstrate that foliar Spray of the Ascophyllum nodosum Algae Extract significant enhanced leaf number compared to the control. The treatment at 5 g L⁻ ¹ exhibited the greatest increase , reaching 5.89 leaves. offshoot⁻ ¹ whereas the control treatment recorded the lowest value of 4.22 leaves offshoot⁻ ¹.

similarly, the data indicate that foliar application of tocopherol had astatistically significant influence on leaf production. The treatment at 140 mg L⁻ ¹ resulted in the highest increase 5.33 leaves offshoot⁻ ¹, in contrast to the control , which showed the lowest increase (4.67 leaves offshoot⁻ ¹).Regarding the interaction between the two factors, the results showed significant differences in the rate of increase in leaf number. The interaction treatment between 5 g L⁻ ¹ of Ascophyllum nodosum and 70 mg L⁻ ¹ of tocopherol resulted in the highest increase (6.33 leaves offshoot⁻ ¹), In contrast, the unteraction (control) recorded the lowest value (3.00 leaves offshoot⁻ ¹)

Results and Discussion:

Table 1. Effect of foliar Spray of the Ascophyllum nodosum Algae Extract and Tocopherol, as well as their interaction, on leaf number (leaves offshoot⁻ ¹) of date palm cv. Saqei.

Ascophyllum nodosum Concentration (g L⁻ ¹)	Tocopherol Concentration (mg L⁻ ¹)			Mean Effect of Ascophyllum nodosum
	0	70	140	
0	3.00	4.00	5.67	4.22
2.5	5.00	4.33	5.00	4.78
5	6.00	6.33	5.33	5.89
Mean Effect of Tocopherol	4.67	4.89	5.33	LSD (0.05) for Ascophyllum nodosum
LSD (0.05) for Tocopherol	0.395			0.395
LSD (0.05) for interaction	0.685			

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The date presented Table (1) clearly indicate that the application of both Ascophyllum nodosum extract and tocopherol significantly improved leaf production compared with the control treatment in date palm cv. Saqei under salinity stress. This increase can be attributed to the fact that seaweed extracts contain various plant growth regulators, such as gibberellins, auxins, and cytokinins, which act as growth stimulators. Additionally, they contain antioxidants, which mitigate the effects of salinity stress, as well as amino acids, macro- and micronutrients, and vitamins [18].

Regarding tocopherol, it is a type of antioxidant that reduces damage caused by high salinity by protecting plant tissues from reactive oxygen species (ROS) generated under salinity stress, either by scavenging or neutralizing them [19]. These findings are consistent with those of [20], who reported similar effects of tocopherol application on date palm cv. Barhi under salinity stress.

Total Chlorophyll (mg 100 g^{-1} fresh weight)

The statistical analysis shown Table(2) revealed significant differences in total chlorophyll content in leaves due to foliar foliar Spray of the Ascophyllum nodosum Algae Extract. the highest chlorophyll level(3.46 mg 100 g^{-1} fresh weight) was recorded in the treatment at 5 g L^{-1}, while the lowest value (2.02 mg 100 g^{-1} fresh weight) was observed in the control treatment.

In contrast, foliar Spray of tocopherol did not produce a statistically significant effect on chlorophyll content. However, the highest value(3.12 mg 100 g^{-1} fresh weight) was recorded at 70 mg L^{-1}, compared with the control treatment, which showed the lowest content (2.70 mg 100 g^{-1} fresh weight). Furthermore significant differences were observed for the interaction between the two factors. The combination of 2.5 g L^{-1} of Ascophyllum nodosum with 70 mg L^{-1} tocopherol resulted in the highest total chlorophyll content (3.98 mg 100 g^{-1} fresh weight), whereas the interaction control treatment recorded the lowest chlorophyll content (1.77 mg 100 g^{-1} fresh weight).

Chemical Traits

Table 2. Effect of foliar Spray of the Ascophyllum nodosum Algae Extract and Tocopherol and their interaction on the Total Chlorophyll (mg 100 g^{-1} fresh weight) of date palm cv. Saqei.

Ascophyllum nodosum Concentration (g L^{-1})	Tocopherol Concentration (mg L^{-1})			Mean Effect of Ascophyllum nodosum
	0	70	140	
0	1.77	1.90	2.39	2.02
2.5	2.87	3.98	3.38	3.41
5	3.44	3.49	3.45	3.46
Mean Effect of Tocopherol	2.70	3.12	3.07	LSD (0.05) for Ascophyllum nodosum
LSD (0.05) for Tocopherol	NS			0.530
LSD (0.05) for interaction	0.919			

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Total Soluble Carbohydrates (%)

The results presented in Table (3) indicated that there were no significant differences in the percentage of total soluble carbohydrates in the leaf with respect to the Ascophyllum nodosum algae extract factor. The total soluble carbohydrate content reached 21.06% at rate 5 g L^{-1}, compared with the treatment control, which recorded 18.20%. Similarly, foliar application of tocopherol did not result in significant differences in carbohydrate content. However, regarding the interaction between the two studied factors, namely Ascophyllum nodosum extract and tocopherol, a significant effect was observed on the percentage of total soluble carbohydrates. The interaction treatment between 5 g L^{-1} of Ascophyllum nodosum and the control treatment showed a significant superiority, recording the highest carbohydrate content of 25.48%, whereas the control interaction treatment showed the lowest leaf content total soluble carbohydrates at 11.94%.

Table 3. Effect of foliar Spray of the Ascophyllum nodosum Algae Extract and Tocopherol and their interaction on theTotal Soluble Carbohydrates (%) of date palm cv. Saqei.

Ascophyllum nodosum Concentration (g L^{-1})	Tocopherol Concentration (mg L^{-1})			Mean Effect of Ascophyllum nodosum
	0	70	140	
0	11.94	18.36	24.32	18.20
2.5	21.07	19.72	21.02	20.60
5	25.48	19.98	17.73	21.06
Mean Effect of Tocopherol	19.50	19.35	21.02	LSD (0.05) for Ascophyllum nodosum
LSD (0.05) for Tocopherol	NS			NS
LSD (0.05) for interaction	5.007			

Proline Amino Acid (μg g^{-1})

The results presented in Table (4) demonstrate the effect of foliar application of Ascophyllum nodosum and tocopherol, as well as their interaction, on the proline content of the leaves.

The data clearly indicate a significant effect of the first factor, namely the algae extract (Ascophyllum nodosum), on leaf proline content. The control treatment recorded the highest mean proline content, reaching 22.01 μg g^{-1}, compared with at rate (5 g L^{-1}) of the extract, showed that the lowest proline content at 14.37 μg g^{-1}. The same results also reveal significant differences in the effect of the second factor (tocopherol) on leaf proline levels. The treatment with 140 mg L^{-1} tocopherol recorded the lowest proline content (15.30 μg g^{-1}), whereas the control treatment recorded the highest value (19.47 μg g^{-1}). In addition, a significant interaction effect between the two experimental factors observed on leaf proline content. The interaction treatment between 2.5 g L^{-1} of Ascophyllum nodosum and 140 mg L^{-1} of tocopherol resulted in the lowest proline content (13.14 μg g^{-1}), whereas the control interaction treatment recorded the highest proline content, reaching 29.82 μg g^{-1} .

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Table 4. Effect of foliar Spray of the Ascophyllum nodosum Algae Extract and Tocopherol and their interaction on the Amino Acid Proline (μg g^{-1}) of date palm cv. Saqei.

Ascophyllum nodosum Concentration (g L^{-1})	Tocopherol Concentration (mg L^{-1})			Mean Effect of Ascophyllum nodosum
	0	70	140	
0	29.82	17.68	18.51	22.01
2.5	14.50	17.89	13.14	15.17
5	14.09	14.77	14.26	14.37
Mean Effect of Tocopherol	19.47	16.78	15.30	LSD (0.05) for Ascophyllum nodosum
LSD (0.05) for Tocopherol	1.740			1.740
LSD (0.05) for interaction	3.013			

The results presented in Tables (2, 3, and 4), as previously shown, demonstrated an increase in both chlorophyll content and carbohydrate levels following the application of Ascophyllum nodosum extract and the antioxidant tocopherol. This increase may be attributed to the role of Ascophyllum nodosum extract and tocopherol in scavenging free radicals, thereby reducing chlorophyll degradation, stabilizing and protecting photosynthetic pigments, and delaying leaf senescence under salinity stress conditions through the protection of chloroplasts from oxidative damage [21,22].

This effect positively contributed to an increase in total soluble carbohydrates in the leaves. These findings are consistent with those reported by [23] in date palm cv. Barhi trees treated with foliar application of tocopherol.The increase in proline content under salinity stress conditions plays an important role in free radical scavenging and osmotic adjustment. This occurs through an increase in cellular osmotic pressure, which enhances the ability of cells to absorb water from adjacent cells, thereby improving stress tolerance [24,25]. However, treatment with Ascophyllum nodosum extract and tocopherol resulted in a reduction in content proline. This reduction may be related to the antioxidants present in Ascophyllum nodosum extract, as well as the role of tocopherol in enhancing photosynthetic activity, promoting carbohydrate formation and stimulating protein synthesis. Proteins constitute the fundamental components of enzymes that mitigate salinity stress, leading to a reduction in proline accumulation. Moreover, salinity stress disrupts protein synthesis and enhances protein degradation, resulting in the inhibition of proline-oxidizing enzyme activity [26].

These results are consistent whit those reported by[29], who found similar effects in date palm trees treated with tocopherol.

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