Impact of Foliar Seaweed and Alpha-Tocopherol Applications on the Morphological and Phytochemical Traits of 'Black Diyala' Figs.

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Abstract

The study was conducted on fig trees (*Ficus carica* L) cv. 'Black Diyala'. This experiment was aimed at supporting the fig crops, as little research is conducted on the use of alpha-tocopherol in this type of cultivation. The study was conducted at a private orchard in Al-Kifl district, south of Babil Governorate, Iraq where six years old trees with even growth and size were chosen. The experiment was prepared as a 3 × 3 factorial in RCBD with three replicates. The first factor was levels of seaweed extract (no spray, 4 g L^{-1} and 4 g L^{-1} combined with calcium at 2 ml L^{-1}) which symbolized as S_1, S_2 and S_3 respectively. The second variable consisted of 3 levels of α-tocopherol, (0; 150; 300 mg L^{-1}) E_1, E_2 and E_3 respectively.

The combined treatment S_3E_3 [Seaweed extract (4 g L^{-1}) + Calcium (2 ml L^{-1}) + alpha-tocopherol (300 mg L^{-1})] was significantly effective for increasing leaf area, leaf dry matter percentage, chlorophyll content, and leaves concentrations of phenolic compounds and flavonoids as well as fruits anthocyanin content compared with the control treatment. On the other hand, S_3E_2 treatment (seaweed extract 4 g L^{-1} + calcium 2 ml L^{-1} with alpha-tocopherol at 150 mg L^{-1}) induced a high number of new shoots.

Keywords: (Alga 600); Antioxidants; Sustainable agriculture; Calcium; Phenolics compounds.

Introduction

Fig (*Ficus carica* L.) is one of the earliest-known fruit trees alongside humans, It is a plant of the genus Ficus in the family Moraceae and it is an important source crop in many countries especially Iraq. The consensus identifies the Arabian Peninsula as the primary geographical source of fig that subsequently disseminated into parts of Asia Minor, extending to such countries as Afghanistan and Turkey. Importantly, Turkey is the largest producer of figs in the world. As reported in the FAO database [16] fig global production amounted to 1,242,449 tons worldwide (total across all countries and different cultivars) at the year of 2022. With 9,681 tons of figs produced per year in Iraq, fig cultivation and productivity is considered relatively poor. Average yield per tree is about 23.45 kg $tree^{-1}$ [13]. There is a reported world-wide movement towards sustainability in agriculture

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globally with the “goal of obtaining food security while maintaining and improving productivity of land, water, and biodiversity. This method is based on environmentally sustainable technologies and practices which can aid in the solution of the most important problems, especially those related to climate change. One of the methods already in use is seaweed extracts used for both soil and plant applications, offering great integration with sustainable development goals against climate change and pollution of soil, water, and plant systems.

One of the most significant organic resources exploited in agricultural production consists of seaweed extracts due to its content of macro- and micronutrients, as well as growth-promoting substances (auxins, cytokinins and vitamins) at various concentrations. Moreover, they also contain essential organic acids, amino acids and polysaccharides that promote a variety of positive effects on the physiological activity of the plant [3]. By the composition of amino acids, low molecular mass carbohydrates including phenolic compounds (due to high concentrations of these components) seaweed extracts stimulate plant physiological functions on one side and also increase activity of soil microorganisms on the other hand [22]. Calcium is an Essential Macronutrient Needed In Large Quantities, It is important for both the development and stability of stiff cell walls, and can furthermore increase the structural strength and quality of roots, stems, leaves and fruits, Calcium has limited mobility through the plant tissue, so foliar application is a way of compensating for deficiency and can ensure sufficient amounts are available during key growth stages [34]. Metabolically, Alpha-tocopherol has also four other roles besides protecting against oxidative stress through endogenous biosynthesis or exogenous application due to its direct effect on transpiration and respiration rates of the leaves [33], which leads to improving plants tolerance response for multiple stresses.

Objectives of the Study

This study investigates the effects of seaweed extracts + calcium and alpha-tocopherol foliar applications on selected vegetative and qualitative parameters of fig trees, as well as phytochemical content.

Material and Methods

During the 2022 growing season, a 3 × 3 factorial experiment was carried out in a randomized complete block design (RCBD). Grown in a private fig orchard located at Al-Kifl district, south of Babil Governorate, Iraq. It consisted two factors were three levels of seaweed exracts (Alga 600) included: S_1, where spraying only water and it was a control; S_2 where the plant was treated with seaweed extract containg 4 g L^{-1}; and S_3 which is leaf application of seaweed extract combined with calcium at 4 g L^{-1} + 2 ml L^{-1}.

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Table 1. Effective ingredients of seaweed (Alga 600).

N	1%
K_2O	17%
Mg	0.42%
S	1%
Organic Matter	35-45
Alganic acid	12- 15
PGR, Vitamine, Acid amine, Manitol, etc.	

Factor two: was comprised of three levels of α- tocopherol were: E_1 (control), E_2 (150 mg L^{-1}) and E_3 (300 mg L^{-1}). There were three replicates, with nine treatments in a randomized arrangement. Each treatment had two trees, leading to an overall tree number of 54 used in this study.

Uniform stand of fig trees with respect to growth and size were selected and planted at a spacing of 6.5 × 5 m between rows and plants. All trees of each treatment received equal pruning while the cultural practices including irrigation, fertilization and pest control application were similar for all treatments equally.

Studied Traits

Leaf and fruit samples were collected to perform the required measurements, including:

Vegetative Growth Traits

leaf area (cm^2)

Which was determined using a digital planimeter (Placom, Japan).

Leaf dry matter (%)

five leaves taken and cleaned then weighed before and after dried, according to equation:

$$\textbf{\% leaf dry} = \frac{\textit{Leaf dry mass}}{\textit{Leaf wet mass}} \textbf{*100} \text{ [9].}$$

Chlorophyll content

Determined by using 0.2 g of leaf powder which extracted with acetone 80%, then measured by spectrophotometer at 663 and 645 nm**,** total Chlorophyll estimated following the equation

Total Chlorophyll (mg/L) = 20.2 D (645) + 8.02 D (663). [19].

Number of new branches

recorded new branches per tree at the finishing the study.

Bioactive compounds

Total phenols:

1 ml of extract which prepared in advance from leaf powder, mixed with 5 ml of Folin argent 10%, then added 4 ml of sodium carbonate 7.5%, after samples incubated for 90 min at 25 C° measured at 750 nm. Calibration curve by using Galic acid at Con. 10-100 mg L^{-1}, content was estimated according to [30].

Total flavonoids:

were determined following the method of [35], 0.5 ml of extract was mixed with 1.5 of methanol, then added 0.1 of $AlCl_3$, and 0.1 of potassium acetate 1M, samples incubation at room temperature, measured at 400 nm. calibration curve by using Catechin at Con. (5-45) µg cm^{-1}.

Tannin content was assessed using the procedure described by [12], by using catechin as standard, then take 400 μl^{-1} of

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extract which added to 3 ml of vanillin and 1.5 of HCl, then measured at 500 nm.

Anthocyanin content

Pigment in fig fruits was determined by using extract of fruits tissue then mixed with (1.5 M) of HCl and Methanol 85%, absorbance measured by Spectrophotometer at 535 nm, according to [15].

Statical Analysis

Data were statistically analyzed using GenStat software, and treatment means were compared using the least significant difference (LSD) test at a probability level of 0.05 [7].

Results and Discussion

Vegetative Growth Traits

Leaf area (cm^2)

The results presented in Table (1) indicate that the foliar application of seaweed extract and α-tocopherol had a significant effect on leaf area, The application of seaweed extract alone resulted in significant increases in leaf area, which **S_3** treatment recorded the highest mean values of 178.18 cm^2, compared with the control treatment **S_1**, which showed the lowest values of 159.72 cm^2.

Similarly, the individual effect of α-tocopherol application, The **E_3** treatment (300 mg L^{-1}) produced the highest mean values of 175.05 cm^2, compared to the control **E_1**, which recorded the lowest values of 164.01 cm^2.

Same The Table indicate that the interaction between foliar application of seaweed extract and α-tocopherol had a significant effect on leaf area. The treatment **S_3E_3**, which involved spraying seaweed extract at (4 g L^{-1}) along with α-tocopherol at 300 mg L^{-1}, recorded the highest mean values of 184.19 cm^2, In contrast, the control treatment **S_1E_1** showed the lowest mean values of 156.43 cm^2

Table 2. Effect of spraying seaweed extract and Tocopherol and their interaction on leaf area (cm^2)

Seaweed	α- Tocopherol			Mean S
	E_1 0 (mg L^{-1})	E_2 150 (mg L^{-1})	E_3 300 (mg L^{-1})	
S_1	156.43	159.17	163.56	159.72
S_2	162.33	171.94	177.41	170.56
S_3	173.27	177.08	184.19	178.18
Mean E	164.01	169.39	**175.05**	
L.S.D $_{0.05}$	**S*E = 7.866**		**E = 4.542**	**S = 4.542**

Number of new branches

The results presented in Table (2) indicate that the foliar application of seaweed extract alone had a significant effect on number of new branches, which **S_3** treatment recorded the highest mean values of 18.67, compared with the control treatment **S_1**, which showed the lowest values of 12.77.

Similarly, the individual effect of α-tocopherol application, The **E_3** treatment (300 mg L^{-1}) produced the highest mean values of 18.11, compared to the control **E_1**, which recorded the lowest values of 13.22.

However, The Table indicate that the interaction between foliar application of seaweed extract and α-tocopherol had a exhibited a significant effect on number of new branches. The treatment **S_3E_2**, which involved spraying seaweed extract at (4 g L^{-1}) along with α-tocopherol at 150 mg L^{-1}, which recorded the highest mean values 20.67, In contrast, the control treatment **S_1E_1** showed the lowest mean values 10.33.

Table 3. Effect of spraying seaweed extract and Tocopherol and their interaction on Number of new branches.

Seaweed	**α- Tocopherol**			**Mean S**
	E_1 0 (mg L^{-1})	**E_2 150 (mg L^{-1})**	**E_3 300 (mg L^{-1})**	
S_1	10.33	12.67	15.33	12.77
S_2	13.67	17.33	19.33	16.77
S_3	15.67	20.67	19.67	18.67
Mean E	13.22	16.89	18.11	
L.S.D $_{0.05}$	**S*E = 2.31**		**E = 1.288**	**S =1.288**

Leaf dry matter (%)

Through the results presented 0f Table (3) it was shown that there was a significantly excelled when spraying of seaweed extract alone had a significant effect on Leaf dry matter (%), which **S_3** treatment recorded the highest mean values of 27.27%, Compared to the no-spray treatment S_1, which gave the lowest value 21.19%.

Similarly, the individual effect of α-tocopherol application, The **E_3** treatment (300 mg L^{-1}) produced the highest mean values of 27.57%, compared to the control

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E_1, which recorded the lowest values of 20.94 %.

However, The Table indicate that the interaction between foliar application of seaweed extract and α-tocopherol had a exhibited a significant effect on number of new branches. The treatment **S_3E_3**, which involved spraying seaweed extract at (4 g L^{-1}) along with α-tocopherol at 300 mg L^{-1}, which recorded the highest mean values 31.14%, In contrast, the control treatment **S_1E_1** showed the lowest mean values 19.20%.

Table 4. Effect of spraying seaweed extract and Tocopherol and their interaction on Leaf dry matter (%)

Seaweed	α- Tocopherol			Mean S
	E_1 0 (mg L^{-1})	**E_2** 150 (mg L^{-1})	**E_3** 300 (mg L^{-1})	
S_1	19.20	21.16	23.22	21.19
S_2	20.29	24.04	28.35	24.23
S_3	23.34	27.32	31.14	27.27
Mean E	20.94	24.17	27.57	
L.S.D $_{0.05}$	**S*E= 3.91**		**E= 2.26**	**S= 2.26**

Chlorophyll content

The results in Table (4) indicate that there is significant difference when spraying of seaweed extract alone, which had a significant effect on Chlorophyll content, the treatment **S_3** gave the highest mean values of 22.65, Compared to the no-spray treatment S_1, which gave the lowest value 16.85.

Similarly, the individual effect of α-tocopherol application, The **E_3** treatment (300 mg L^{-1}) produced the highest mean values of 21.23, compared to the control **E_1**, which recorded the lowest values of 18.42.

However, The Table indicate that the interaction between foliar application of seaweed extract and α-tocopherol had a exhibited a significant effect on number of new branches. The treatment **S_3E_3**, which involved spraying seaweed extract at (4 g L^{-1}) along with α-tocopherol at 300 mg L^{-1}, which recorded the highest mean values 23.78, In contrast, the control treatment **S_1E_1** showed the lowest mean values 15.13.

Table 5. Effect of spraying seaweed extract and Tocopherol and their interaction on Chlorophyll content

Seaweed	**α- Tocopherol**			**Mean S**
	E_1 0 (mg L^{-1})	**E_2 150 (mg L^{-1})**	**E_3 300 (mg L^{-1})**	
S_1	15.13	16.78	18.63	16.85
S_2	18.62	20.09	21.27	19.99
S_3	21.51	22.65	23.78	22.65
Mean E	18.42	19.84	21.23	
L.S.D $_{0.05}$	**S*E= 1.95**		**E= 1.12**	**S= 1.12**

The observed increases may be attributed to the role of enzymes, chlorophyll, essential amino acids, and vitamins (C, B, and E) present in seaweed extracts, which promote cell division and elongation, enhance vegetative growth traits, and improve the plant's capacity to synthesize and accumulate assimilates. In addition, seaweed extracts are rich in nitrogen, phosphorus, and potassium, which play a crucial role in increasing leaf area, enhancing photosynthetic activity, and boosting carbohydrate production, ultimately leading to improved vegetative growth [10].

[26] reported that foliar application of such extracts enhances nutrient uptake efficiency, reduces alternate bearing in apple trees, and increases leaf chlorophyll content through improved photosynthesis and respiration processes. These findings are consistent with those reported by [4,6], who indicated that seaweed extract application significantly improves metabolic processes and increases photosynthetic products, resulting in higher leaf dry matter percentage and greater numbers of new shoots [25]. Similar results were also documented by [8] in apricot, [17] in strawberry and [29] in fig trees.

Calcium application enhances cell division and elongation, thereby promoting root growth and improving nutrient uptake by plants [14]. Furthermore, antioxidants play a significant role in improving plant growth by stimulating enzymatic activity, which enhances photosynthetic efficiency, nutrient absorption, and overall nutritional

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status, leading to improved vegetative growth [2,20]. This effect may also be attributed to their role in improving the photosynthetic system by reducing the production of reactive oxygen species (ROS) [27]. Consequently, this was reflected in increased branch number, leaf area, and leaf dry matter content. These results are in agreement with those reported by [21].

Bioactive compounds
Total phenols

The results presented in Table (5) indicate that the foliar application of seaweed extract and α-tocopherol had a significant effect on Bioactive compounds, The application of seaweed extract alone resulted in significant increases in Total phenols, which **S_3** treatment recorded the highest mean values of 147.49 mg g^{-1}, compared with the control treatment **S_1**, which showed the lowest values of 132.19 mg g^{-1}.

Similarly, the individual effect of α-tocopherol application, The **E_3** treatment (300 mg L^{-1}) produced the highest mean values of 130.86 mg g^{-1}, compared to the control **E_1**, which recorded the lowest values of 151.42 mg g^{-1}.

Regarding The interaction between foliar application of seaweed extract and α-tocopherol, Table (5) indicate that the had a significant effect on Total phenols. The treatment **S_3E_3**, which involved spraying seaweed extract at (4 g L^{-1}) along with α-tocopherol at 300 mg L^{-1}, recorded the highest mean values of 158.62 mg g^{-1}, In contrast, the control treatment **S_1E_1** showed the lowest mean values of 123.80 mg g^{-1}.

Table 6. Effect of spraying seaweed extract and Tocopherol and their interaction on Total phenols mg g^{-1}

Seaweed	**α- Tocopherol**			**Mean S**
	E_1 0 (mg L^{-1})	**E_2** 150 (mg L^{-1})	**E_3** 300 (mg L^{-1})	
S_1	123.80	137.12	135.64	132.19
S_2	134.83	144.86	160.00	146.56
S_3	133.96	149.90	158.62	147.49
Mean E	130.86	143.96	151.42	
L.S.D $_{0.05}$	**S*E= 1.40**		**E= 0.81**	**S= 0.81**

Total flavonoids

The results presented in Table (6) indicate that the foliar application of

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seaweed extract and α-tocopherol had a significant effect on Bioactive compounds, The application of seaweed extract alone resulted in significant increases in Total flavonoids, which **S_3** treatment recorded the highest mean values of 28.01mg g^{-1}, compared with the control treatment **S_1**, which showed the lowest values of 23.61mg g^{-1}.

Similarly, the individual effect of α-tocopherol application, The **E_3** treatment (300 mg L^{-1}) produced the highest mean values of 27.95 mg g^{-1}, compared to the control **E_1**, which recorded the lowest values of 24.14 mg g^{-1}.

Regarding The interaction between foliar application of seaweed extract and α-tocopherol, Table (5) indicate that the had a significant effect on Total phenols. The treatment **S_3E_3**, which involved spraying seaweed extract at (4 g L^{-1}) along with α-tocopherol at 300 mg L^{-1}, recorded the highest mean values of 27.95 mg g^{-1}, In contrast, the control treatment **S_1E_1** showed the lowest mean values of 22.54 mg g^{-1}.

Table 7. Effect of spraying seaweed extract and Tocopherol and their interaction on Total flavonoids

Seaweed	**α- Tocopherol**			**Mean S**
	E_1 0 (mg L^{-1})	**E_2 150 (mg L^{-1})**	**E_3 300 (mg L^{-1})**	
S_1	22.54	23.18	25.11	23.61
S_2	24.11	28.34	27.96	26.80
S_3	25.79	27.46	30.78	28.01
Mean E	24.14	26.32	27.95	
L.S.D $_{0.05}$	**03S*E= 2.**		**E= 1.01**	**S= 1.01**

Tannin (%)

Through the results presented 0f Table (7) it was shown that there was a significantly excelled when spraying of seaweed extract alone had a significant effect on tannin, which **S_3** treatment recorded the highest mean values of 0.58 %, Compared to the no-spray treatment S_1, which gave the lowest value 0.4 %.

Similarly, the individual effect of α-tocopherol application, The **E_3** treatment (300 mg L^{-1}) produced the highest mean values of 0.53 %, compared to the control **E_1**, which recorded the lowest values of 0.49 %.

However, The Table (7) indicate that the interaction between foliar application of seaweed extract and α-tocopherol had a exhibited a significant effect on tannin. The

ISSN 2072-3857

treatment S_3E_3, which involved spraying seaweed extract at (4 g L^{-1}) along with α-tocopherol at 300 mg L^{-1}, which recorded the highest mean values 0.61%, In contrast, the control treatment S_1E_1 showed the lowest mean values 0.42 %.

Table 8. Effect of spraying seaweed extract and Tocopherol and their interaction on tannin (%)

Seaweed	α- Tocopherol			Mean S
	E_1 0 (mg L^{-1})	E_2 150 (mg L^{-1})	E_3 300 (mg L^{-1})	
S_1	0.42	0.43	0.47	0.44
S_2	0.5	0.49	0.52	0.5
S_3	0.55	0.57	0.61	0.58
Mean E	0.49	0.5	0.53	
L.S.D $_{0.05}$	**S*E= N.S**		**E= 0.038**	**S= 0.038**

Anthocyanin content (µg g^{-1})

It is clear from the results of Table (8) which indicate that the foliar application of seaweed extract alone had a significant effect on Anthocyanin content, which S_3 treatment gave the highest mean values of 71.76 µg g^{-1}, compared with the control treatment S_1, which showed the lowest values of 58.9 µg g^{-1}.

Similarly, the individual effect of α-tocopherol application, The E_3 treatment (300 mg L^{-1}) produced the highest mean values of 82.45 µg g^{-1}, compared to the control E_1, which recorded the lowest values of 49.57 µg g^{-1}.

However, The Table indicate that the interaction between foliar application of seaweed extract and α-tocopherol had a exhibited a significant effect Anthocyanin content. The treatment S_3E_2, which involved spraying seaweed extract combined at (4 g L^{-1}) along with α-tocopherol at 300 mg L^{-1}, which recorded the highest mean values 72.7 µg g^{-1}, In contrast, the control treatment S_1E_1 showed the lowest mean values 58.04 µg g^{-1}.

ISSN 2072-3857

Table 9. Effect of spraying seaweed extract and Tocopherol and their interaction on on Anthocyanin content

Seaweed	α- Tocopherol			Mean S
	E_1 0 (mg L^{-1})	E_2 150 (mg L^{-1})	E_3 300 (mg L^{-1})	
S_1	49.57	64.71	62.43	58.9
S_2	62.25	60.68	73.21	65.38
S_3	62.31	70.52	82.45	71.76
Mean E	58.04	65.31	72.7	
L.S.D $_{0.05}$	**S*E= 2.04**		**E= 1.17**	**S= 1.17**

Foliar application of seaweed extract significantly increased the content of bioactive compounds, particularly phenolics and flavonoids, in fig leaves. This increase can be attributed to the rich composition of seaweed extracts, which include polysaccharides, vitamins, antioxidants, and secondary metabolites such as phenolic compounds that contribute to protecting plant cells and their components [23]. In addition, seaweed extracts enhance the nutritional status of plants by increasing the availability and uptake of essential nutrients, particularly nitrogen, phosphorus, and potassium [5], which in turn significantly improves the accumulation of bioactive compounds in leaves [11]. These findings are consistent with those reported by [24].

Furthermore, calcium ions play a crucial role in stimulating the production of reactive oxygen species (ROS) through activation of NADPH oxidase, which enhances the activity and efficiency of antioxidant enzymes and promotes the synthesis of phenolic compounds [31]. The enzyme phenylalanine ammonia-lyase (PAL), a key enzyme in the phenylpropanoid pathway responsible for flavonoid biosynthesis, is also regulated by calcium via the activation of calmodulin (CaM) and other calcium-binding proteins [32]. These results are in agreement with those reported by [18].

Alpha-tocopherol plays a vital physiological role in promoting plant growth, enhancing drought tolerance, and reducing leaf abscission by stimulating the activity of key enzymes involved in photosynthesis and protecting cellular membranes. Moreover, it acts as a potent antioxidant [1]. These findings are consistent with those reported by [21] in fig trees and [28] in mango.

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