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Effect of Salinity and Spraying of *Spirulina* sp. Extract on Some Morphological and Physiological Characteristics of Anise (*Pimpinella anisum* L.)

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Abstract

The experiment was conducted during the academic year (2023–2024) in the Al-Ghammas area, affiliated with Al-Shamiya District/Diwaniyah Governorate, with the aim of studying the effect of salinity of irrigation water and spraying Spirulina plantensis algae extract on some phenotypic and physiological growth characteristics of the anise plant Pimpinella anisum which were measured in the laboratories of the Department of biology/College of Education for women – University of Kufa, and the experiment was implemented using a Randomized Complete Block Design (R.C.B.D) in three replicates with the presence of two factors, which are the salt concentrations (0, 2 and 5) ds.m^{-1} and the algal extract concentrations (0, 5 and 10) ppm. The averages were compared according to Duncan's multi-range test at the probability level of 0.05, and the results showed that the salinity of irrigation water led to a decrease in most of the studied growth characteristics of the plant except for the content of the amino acid proline in the leaves, which recorded an increase in rates as its levels increased. Also, spraying the algal extract, especially at a concentration of 10 ppm, led to improving the characteristics and reducing the negative effect of salinity, the increased salinity also led to an increase content of the amino acid proline of leaves, which plays a major role in reducing salt stress and its effect on the plant and Spraying plants with levels of algae extract improved the studied plant characteristics and reduced the severity of salt stress on plants. Medicinal plants in soil are affected by salts, including studying morphological and biochemical characteristics to serve as a guide for evaluating these plants in their ability to tolerate high concentrations of salinity and the necessity of using algal extracts of different species as fertilizer supplements for plants.

Keywords: *Pimpinella anisum*, salt stress, *Spirulina* sp, proline, Chlorophyll

INTRODUCTION

Anise (*Pimpinella anisum* L.) is an annual herbaceous plant belonging to the Apiaceae family with white flowers and small green to yellow seeds [1]. Anise has been used as a spice, flavoring, and aromatic substance, and its seed extracts and oils have been used since ancient times in many therapeutic recipes of folk medicine in Arab countries, including Iraq [2]. Anise seeds contain many active compounds, the most important of which are anethole, anisaldehyde, methylthiacol, alkaloids, proteins, etc. [3]. Anise is also used as an appetite stimulant, nerve sedative, and diuretic. It also has many therapeutic effects on many respiratory, digestive, nervous, and gynecological disorders [4]. Increasing land productivity to meet the world's growing demands and needs for plants is an important priority. Environmental stresses are also among the main determining factors for plant production and productivity. Salt stress is one of these stresses that programs and research still focus on. Salt stress has a wide impact on plants, as it greatly affects the early stages of the plant

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(germination – seedling). The vegetative stages are very sensitive as it affects the accumulation of dry matter, the chlorophyll content of leaves, stem elongation and leaf area. The effects of salt stress vary according to the age of the plant, the type of organs and the concentration of salts [5]. Foliar feeding is one of the most efficient fertilization methods, as it supplies nutrients to the plant when there is a problem in absorbing elements from the soil [6]. Blue–green algae are a natural source of many plant hormones such as auxins, gibberellins, cytokinins, organic matter, and mineral macro- and micronutrients. *Spirulina plantensis* is one of the members of this phylum [7, 8] in addition to containing many vitamins, proteins, and some amino acids that improve plant growth and productivity [8, 9]. *Spirulina* sp. has positive effects in improving plant growth, encouraging root growth and formation, and increasing the efficiency of photosynthesis [10]. It also plays an important role in stimulating flower bud development and reducing leaf, flower, and fruit drop [11]. It also increases the vegetative growth period and delays aging, in addition to its use as an antioxidant agent, as it helps the plant withstand stress conditions [12–14].

The research aims to study the effect of different concentrations of sodium chloride salt (NaCl) on the growth of anise plant and its morphological and physiological characteristics, in addition to trying to reduce the damage of salinity on these studied characteristics by using *Spirulina* algae extract sprayed on the plant's vegetative system to improve its growth as an efficient fertilization method to eliminate the fixation of nutrients in the soil, since the soil of Iraq is alkaline.

MATERIALS AND WORKING METHODS

The seeds were planted directly in the field on 11/11/2023 (Figure 1) at a rate of 6 seeds per hole, and the experiment continued until 1/15/2024. Salinity concentrations (0, 2, and 5) dsm^{-1} were prepared in decisiemens (DSM) according to the equation $dsm^{-1} = ppm \div 640$, as reported in the study of Jazdan and Omar (2010) [15]. Salts were added to the irrigation water, and the method described by Ling and Silberbush (2002) [16] was used. To obtain the algal extract, distilled water and a continuous extraction device (Soxhlet extractor) were used. Dry quantities of algae, about 5 gm, were placed in the extraction device with 200 ml of distilled water at a temperature of 80°C for 4 hours. The extract was stored in an opaque glass flask and placed in the refrigerator until use. Concentrations of the algal extract were prepared (0, 5 and 10) ppm. The plants were sprayed until completely wet using a hand sprayer. Fifteen (15) days after transplanting in the field, spraying was repeated every 15 days. Tween-20 (0.15 cm^3/L^{-1}) was added as a spreading agent [16]. The average leg length was measured using a graduated ruler for three repetitions. The average root length was measured using a graduated ruler for three repetitions [17]. The number of branches per plant was also calculated as an average of three repetitions. The samples used to measure the fresh weight were dried in an electric oven at a temperature of 65°C until the weight was constant. The dry weight was calculated using a sensitive balance [18]. Salt stress tolerance index was measured according to the method of Meneguzzo et al. (2000) [19]. The average content of chlorophyll in the leaves was taken for three replicates of each treatment, and this was done using a chlorophyll meter, type SPAD-502 [20]. The method of Morales-Payan and Norrie (2010) [21] was also followed in estimating the proline content of leaves.

RESULTS AND DISCUSSION

Tables 1–7 indicate the effect of irrigation with saline water and foliar spray treatment with *spirulina* extract on the following properties: stem height rate, root length, number of branches, guide to salt tolerance, plant dry weight, total Chlorophyll content of leaves and Proline content of leaves, as the salt concentration of 5 dsm^{-1} recorded a significant decrease in the above characteristics, Except for the leaf content of the amino acid proline, which recorded high rates with increasing salt and the rates reached (15.01 cm, 5.03 cm, 3.08 branches, 0.74, 3.77 gm, 2.48 spad and 2.48 $Mm.gm^{-1}$), respectively, compared with the comparison treatment recorded the highest rates (15.01 cm, 5.03 cm, 3.08 branches, 0.74, 3.77 gm, 2.48 spad and 0.62 $Mm.gm^{-1}$), respectively, while the addition of *spirulina* extract led to a noticeable improvement in the studied traits by increasing its concentrations. The highest rates were reached at a concentration of 10 ppm (24.69 cm, 10.12 cm, 5.38 N.b, 0.90, 8.47 gm, 4.69 spad), respectively, while the highest rate of proline was recorded in the comparison treatment to reduce salt stress and amounted to (1.62 $Mm.gm^{-1}$).

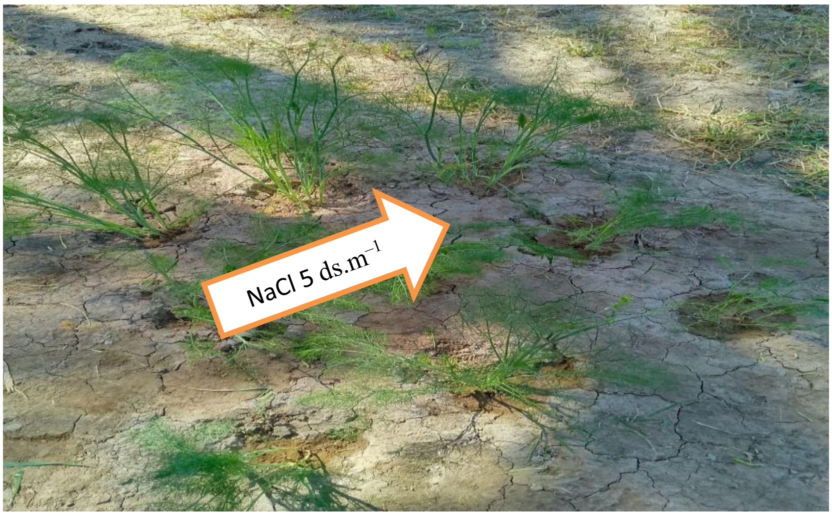

Figure 1. Experiment design in the field.

As the interaction between the two factors, *Spirulina* extract spray led to reducing the effects of salinity in all treatments when interacting (5 dsm^{-1} NaCl + 10 ppm *Spirulina* extract). Except for the proline content of the leaves, which recorded the highest rate in the high salt treatment and the control without adding the extract, it reached (0.84 $Mm.gm^{-1}$).

Table 1. Effect of salinity concentrations and *Spirulina* extract and their interaction on stem height.

Mean	***Spirulina* Extract ppm**			NaCl $ds.m^{-1}$
	10	***5***	***0***	
25.38 a	29.82 a	26.33 b	20.00 c	0
20.17 b	25.31 c	21.00 d	14.22 h	2.5
15.01 c	18.96 f	15.65 g	10.42 i	5
	24.69 a	20.99 b	14.88 c	Mean

Note: Rates with similar letters do not differ from each other within the main factors or their binary interactions according to Duncan's multinomial test at a probability level of 0.05.

Table 2. Effect of salinity concentrations and *Spirulina* extract and their interaction on root length.

Mean	***Spirulina* Extract ppm**			NaCl $ds.m^{-1}$
	10	***5***	***0***	
10.32a	13.20 a	10.32b	7.45f	0
7.64b	9.40c	8.55d	4.99h	2.5
5.03c	7.77e	5.44g	2.25i	5
	10.12a	8.10b	4.89c	Mean

Note: Rates with similar letters do not differ from each other within the main factors or their binary interactions according to Duncan's multinomial test at a probability level of 0.05.

Table 3. Effect of salinity concentrations and *Spirulina* extract and their interaction on number branches.

Mean	***Spirulina* Extract ppm**			NaCl $ds.m^{-1}$
	10	***5***	***0***	
5.13a	6.54a	4.91c	3.95f	0
3.95b	5.10b	4.20e	2.55h	2.5
3.08c	4.52d	3.00g	2.00i	5
	5.38a	4.03b	2.83c	Mean

Note: Rates with similar letters do not differ from each other within the main factors or their binary interactions according to Duncan's multinomial test at a probability level of 0.05.

Table 4. Effect of salinity concentrations and *Spirulina* extract and their interaction on guide to salt tolerance.

Mean	***Spirulina* Extract ppm**			NaCl ds.m^{-1}
	10	***5***	***0***	
0.92a	0.99a	0.93b	0.85c	2.5
0.74b	0.81c	0.77d	0.66e	5
	0.90a	0.85b	0.75c	Mean

Note: Rates with similar letters do not differ from each other within the main factors or their binary interactions according to Duncan's multinomial test at a probability level of 0.05.

Table 5. Effect of salinity concentrations and *Spirulina* extract and their interaction on dry weight.

Mean	***Spirulina* Extract ppm**			NaCl ds.m^{-1}
	10	***5***	***0***	
7.34a	9.22 a	7.35c	5.45f	0
6.18b	8.34b	6.54e	3.66g	2.5
3.77c	7.87d	3.45h	1.99i	5
	8.47a	5.78b	3.70c	Mean

Note: Rates with similar letters do not differ from each other within the main factors or their binary interactions according to Duncan's multinomial test at a probability level of 0.05.

Table 6. Effect of salinity concentrations and *Spirulina* extract and their interaction on content of chlorophyll total.

Mean	***Spirulina* Extract ppm**			NaCl ds.m^{-1}
	10	***5***	***0***	
4.33a	5.88 a	4.62b	2.51f	0
3.39b	4.33c	3.99d	1.87h	2.5
2.48c	3.87e	2.13g	1.45i	5
	4.69a	3.58b	1.94c	Mean

Note: Rates with similar letters do not differ from each other within the main factors or their binary interactions according to Duncan's multinomial test at a probability level of 0.05.

Table 7. Effect of salinity concentrations and *Spirulina* extract and their interaction on content of proline.

Mean	***Spirulina* Extract ppm**			NaCl ds.m^{-1}
	10	***5***	***0***	
0.62c	0.19g	0.20g	0.23f	0
1.47b	0.43e	0.49d	0.55c	2.5
2.16a	0.67b	0.65b	0.84a	5
	1.29b	1.34b	1.62a	Mean

Note: Rates with similar letters do not differ from each other within the main factors or their binary interactions according to Duncan's multinomial test at a probability level of 0.05.

The results obtained in Tables 1–5 can be explained, respectively, by a decrease in stem height, root length, number of branches and dry weight of the vegetative system because of irrigating the plants with water. Salty and increasing concentrations, this is attributed to the fact that the increase in the concentration of salts in irrigation water, which leads to an increase in the osmotic potential in the soil solution within the root zone and a decrease in the water potential, then the difference in the gradient in the water potential between the soil solution and the cells of the root system decreases, which leads to a decrease in the amount of free water with what it contains of mineral elements available to the plant, so the absorption rate decreases. Water, while the plant continues to lose water by transpiration, the amount of lost water becomes greater than the amount absorbed, so the leaf cells are exposed to water deficit and the filling effort inside the leaf cells declines, which explains the decline in the growth and development of the green parts and the decrease in the rate of stem height and the number of branches, which negatively affects the rate of production and accumulation of dry matter, so the available amount decreases during the stages of plant growth, and this is consistent with the study of Naqi et al. (2025), Nawrocka et al. (2017), Osman et al. (2016), and Qasmo (2003) [22–25]. The inhibition and decrease in the efficiency and speed of the photosynthesis process because of salts is due to the decrease in the content of chlorophyll pigments in the leaves (Table 6) as with the increase in the concentration of dissolved ions in the soil solution, it leads to a decline in leaf growth and a decrease in the area of the green leaf surface effective in the photosynthesis process due to the decrease in the amount of light energy absorbed as well as the decrease in nitrogen because of the decrease in the formation of amino acids. These results are consistent with what researchers [26–28] have reached regarding the negative effect of salinity on the formation of plant pigments. Increasing salt concentrations, especially at 5 dS-1, led to a decrease in the chlorophyll content of leaves due to the increased toxicity of sodium ions, which inhibits the activity of the enzymes responsible for building and forming pigments and the development of plastids, and distorts the grana plates that carry the chlorophyll pigment. This is accompanied by the replacement of potassium in the plastids with sodium, and the increased salts decompose chlorophyll and slow down the rate of its formation due to the increase in the plant hormone (abscisic acid ABA), which accelerates the decomposition of chlorophyll pigments due to the lack of sufficient quantities of nitrogen and the low activity of the nitrate reductase enzyme. As the amino acid proline (Table 7), the relationship between the increase in salts and its content inside the plant is a direct relationship. The increasing salinity causes an increase in the accumulation of the amino acid proline inside the plant, as it has an important role in the process of osmotic regulation during salt stress and reducing it [29, 30], while the use of high concentrations of algal extract has yielded positive results in increasing and improving the growth characteristics studied above and reducing the impact of salt stress and its danger to the plant. These results are consistent with the findings of Usharani et al. (2012) and Wahid & Close (2007) [31, 32]. The positive effect of spraying algae extract on most of the vegetative growth characteristics is attributed to its containing many nutritional elements that play an important role in increasing the metabolic activities of the plant, including potassium, which works to activate the enzymes that manufacture amino acids and proteins, as well as it helps in the production of chlorophyll, which is necessary in the process of photosynthesis and the formation of proteins, sugars and energy compounds ATP, which leads to increased plant growth and size, and thus increased vegetative growth and improved studied traits [33].

CONCLUSIONS AND RECOMMENDATIONS

From the results obtained, we conclude the following: The increased salinity in the plant growth medium had a negative impact on some phenotypic and physiological characteristics of the plant, especially at a concentration of 5 dsm^{-1}, which gave a significant difference from the rest of the treatments, the increased salinity also led to an increase content of the amino acid proline of leaves, which plays a major role in reducing salt stress and its effect on the plant and Spraying plants with levels of algae extract improved the studied plant characteristics and reduced the severity of salt stress on plants, especially at a concentration of 10 ppm. As *Spirulina* rich in hormones, such as auxins, cytokinins, and gibberellins, as well as containing nutrients and amino acids, it plays a role in reducing the effects of oxidative stress resulting from the increase of free radicals in the plant. Based on the above, we recommend the following: Conducting field studies and applied research on other medicinal plants in soils affected by salts, including studying morphological and biochemical characteristics to serve as a guide for evaluating these plants in their ability to tolerate high concentrations of salinity and the necessity of using algal extracts of different species as fertilizer supplements for plants, in addition to reducing pollution, as they are safe nutrients for the environment and humans, and the interaction between other study factors.

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