

Effect of Using Algae Extracts on Growth of *Ruellia simplex* Plants Grown under Salinity Stress

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Abstract: The experiment was carried out in the nursery of Antoniadis Garden, Horticulture Research Institute, Alexandria branch, Ministry of Agriculture, Egypt, during the successful growing seasons of 2021 and 2022 (from March 1st to October 30th of each season) to study the effects of irrigated with diluted seawater added directly to medium and foliar applications of biostimulant material (seaweed extract *Ascophyllum Nodosum*) by spraying the foliage on the growth of *Ruellia simplex* L. plant. The seaweed extract *Ascophyllum nodosum*, which was added to distilled water in three concentrations (0.0, 2.0, and 4.0 ml/l) and fresh water was used to dilute samples of Mediterranean water from the city of Alexandria to the required concentrations of 0, 5, 10, and 15% salt water. The design of the experiment was a factorial experiment in a randomized complete blocks design. The study included 12 treatments [4 rates of saline water irrigation x 3 doses of Stimplex extract (ANE)] with three replicates. Each experimental unit contained 4 plants. The data were subjected to analysis of variance (ANOVA) using the SAS program. At the end of the experiment, the results of the statistical analysis showed that the treatment with 4 ml/l of algae extract was superior in terms of main plant height (cm), number of branches, number of leaves, total fresh weight, total dry weight, fresh root weight, dry root weight, number of flowers, days A to Z (days) and chemical composition N%, P%, K%, Ca%, carbohydrate, MSI%. The results of the statistical analysis also showed that the treatment with saline solution had a significant effect on all the characteristics, as the treatment 15.0% lowest value. except Na% concentration. The results of the statistical analysis of the experiment also showed that there were significant interactions between the two experimental factors, the values of which varied according to the characteristics studied.

Key words: Mexican-petunia % *Ruellia simplex* % Algae extracts % Salinity stress % Growth % Flowering % chemical composition

INTRODUCTION

There are 250 species of perennial herbs, subshrubs, and shrubs in the genus *Ruellia* (Acanthaceae), most of which are found in tropical and subtropical regions. Mexican-petunia a (*Ruellia simplex*), known for its profusion of purple flowers that bloom under a variety of conditions, is a perennial herbaceous plant often used in landscaping. Mexican-petunia, a plant native to Mexico and South America, was probably imported as an ornamental plant [1].

Saline water, such as seawater has been considered unsuitable for plant irrigation, but recently, it has been used for irrigation under certain conditions [2]. Salinity

stress causes many changes in different metabolic and biochemical processes in plant cells, depending on the severity and the duration of this stress, ultimately leading to a decline of different crop production. Salinity is one of the effects that suppress plant growth [3-5].

The use of seaweed extracts has become an attractive fertilizer and biostimulants option. Liquid extracts obtained from seaweeds have gained importance as foliar sprays or soil drenches for several crops to stimulate growth and yield, develop environmental stress tolerance, increase nutrient uptake from the soil, and enhance antioxidant properties [6]. The extract contains growth-promoting hormones, cytokines, auxins, trace elements, vitamins, and amino acids [7, 8].

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Table 1: The physical and chemical properties of the soil mixture used in Antoniadis for the two seasons.

pH	EC Ds/m	Cations (meq/l)				Anions(meq/l)		
		Ca ⁺⁺	Mg ⁺⁺	Na ⁺⁺	K ⁺	HCO ₃ ⁻	CL ⁻	SO ₄ ⁻
8.08	2.53	18.20	14.20	23.91	4.49	7.20	21.00	27.10
Soil particles	Clay	Slit			Sand	Soil texture		
%	54.93	16.78			28.29	Clay sandy loam		

Table (2-A): PH and EC values of the different diluted seawater treatments at the start of the experiment.

Seawater (%)	pH	EC (ds/m)
0	7.80	1.49
5	7.31	10.36
10	7.04	13.38
15	7.20	15.48

Table (2-B): Content of simplex *Ascophyllum nodosum* extract.

Mineral							
Content	N (total)	P ₂ O ₅	K ₂ O	S	Mg	Ca	
%	0.6	0.10	0.10	0.2-0.4	0.03-0.1	0.01-0.12	

Of these, using natural seaweeds as a fertilizer has allowed the gradual replacement of conventional synthetic fertilizers [9]. These natural fertilizers are biodegradable, non-toxic, non-polluting, and safe for humans, animals and birds [10]. Seaweeds are macroscopic marine algae. They are used as food for humans, fodder for cattle, a substitute for chemical fertilizer, and a source of various fine chemicals. They are also used to produce many industrial products such as agar and alginat [11].

In recent years, natural seaweed has been used as a substitute for synthetic fertilizers. Seaweed extracts are marketed as liquid fertilizers and bio-stimulants because they contain several growth regulators such as cytokinins, auxins, gibberellins and various macro and micronutrients necessary for plant growth and development [12-14]. Moreover, it helps in promoting the growth of beneficial soil microorganisms Khan *et al.* [11], increase nutrient uptake from the soil and enhancing antioxidant properties [11, 15, 16]. In recent years, the use of seaweed extracts has gained popularity due to their potential use in organic and sustainable agriculture, especially in rainfed crops, as a means to avoid excessive fertilizer applications and to improve mineral absorption and because of their organic and bio-degradable properties [17, 18].

Stimplex, a commercial product made of *A. nodosum* extract, is widely used as an effective and novel formulation of seaweed extract-based biostimulants [19]. *Ascophyllum Nodosum* is known as "rockweed" and grows in great abundance along the coasts of Europe and the Northeast of North America [20, 21].

MATERIALS AND METHODS

A pot experiment was carried out in the nursery of Antoniadis Garden, Horticulture Research Institute, Alexandria branch, Ministry of Agriculture, Egypt, during the successful growing seasons of 2021 and 2022 (from March 1st to October 30th of each season) to study the effects of irrigated of diluted seawater added directly to medium and foliar applications of biostimulant material (seaweed extract *Ascophyllum Nodosum*) by spraying the foliage on the growth of *Ruellia simplex L.*

Plant material and procedure: For the cultivation of *Ruellia simplex L.*, approximately 20cm-long stem cuttings were planted in containers filled with in clay-sandy-loamy soil collected from the nursery Antoniadis Research Gardens on March 1, 2021, and 2022. The chemical constituents of the soil were measured as described by Jackson [22] and are presented in Table (1). Once the cuttings were rooted, they were transplanted into 20cm-diameter plastic containers. The first salinity treatment was applied to the plants three weeks after transplantation.

Water Salinity: Fresh water was used to dilute samples of Mediterranean water from the city of Alexandria to the required concentrations of (0, 5, 10, and 15%) of saline water before determining the EC value, and the pots received 300ml of appropriately diluted seawater every three days, from one month after transplantation until the end of the study, and tap water as a control.

Once every three weeks, the plants were irrigated with fresh tap water to wash out the salt. In the first and second seasons, the tap water had salinity EC values of about 0.47 and 0.42 (dsmG⁻¹), respectively. The chemical properties of seawater and tap water are shown in Table (1), and the pH and EC of the diluted seawater used for irrigation are shown in Table (2-A).

Preparation Seaweed Extracts:

Seaweed Extract and Treatment Preparation:

Seaweed extract (SWE) solution has commercial name is (Stimplex) which contains some component [total N (0.6%) - P₂O₅ (0.10%) - K₂O (0.10%) - S (0.2-0.4%) - MgO

(0.03-0.10%) - Ca (0.01-0.12) (Table 2-B) and other microelements] biostimulant material (seaweed extract *Ascophyllum nodosum*) (ANE) which was added in distilled water at three concentrations (0.0, 2.0, and 4.0 ml/l). the control plants were sprayed with distilled water. As a surfactant. Plants were sprayed three times with different amounts of SWE; the first time after thirty days, the second time after sixty days, and the third time after ninety days from the start of the study. The experiment was repeated three times with four plants per treatment. A control group was exposed to no biostimulants and 0.0% salinity. The extract solution was applied as a foliar spray to the aerial parts. ANE was sprayed three times a month throughout the growing season until the run-off point after transplanting (before the reproductive stage). Regular agricultural activities were carried out as required by the plants.

Data Recorded: At the end of October for each season, the following data were recorded:

Vegetative Growth: Plant height (cm), number of branches/plants, total vegetative fresh weight (g), total dry weight (g), and number of leaves /plants. Moreover, the fresh and dry weights (g) of the roots were recorded in both seasons.

Root Characters: The fresh and dry weights (g) of the roots were measured.

Flowering Parameters: The number of days (days) between transplantation and full flowering for each plant, the total number of flowers produced by each plant during the period of the experiment (up until its termination), and the fresh and dry weights of the flowers (in grams) were recorded as flowering parameters.

Chemical Constituents:

- C Total nitrogen, phosphorus and potassium in the leaves were determined by distillation according to the micro-Kjeldahl method described by Allen [23]; Jackson [24] and Champman and Pratt [25].
- C Calcium and sodium were estimated in leaves.
- C The proline content (% of dry matter) of the leaves was determined according to Bates *et al.* [26].
- C Chlorophyll content was determined as SPAD units in the fresh leaves of the plants for the different experimental treatments at the end of the study using a chlorophyll meter (SPAD) according to Yadava [27].

- C The carbohydrate content (mg/100g D.W.) of the leaves was determined according to Dubois *et al.* [28]. Total carbohydrate content was measured in dry samples according to Herbert *et al.* [29].
- C Furthermore, the content of the free amino acid proline was determined as (mg/g D.W.) according to the method described by Bates *et al.* [26].
- C The Na content was determined as mg/g D.W. [30].
- C The membrane stability index (MSI%) was determined according to Sairam *et al.* [31].
- C Leaf tissue was placed in test tubes containing ten milliliters of double-distilled water [32]. One sample was heated in a water bath at 40EC for 30 minutes and a conductivity bridge (EC₁) was used to record the solution's electrical conductivity. The conductivity of the second sample was measured after boiling for ten minutes at 100°C (EC₂). The following formula was used to determine the MSI%:

$$\text{MSI (\%)} = [1 - (\text{EC}_1 / \text{EC}_2)] \times 100$$

Statistical Analysis: The experimental design was a factorial experiment in a randomized complete block design. The study included 12 treatments [4 rates of saline irrigation x 3 doses of Stimplex extract (ANE)] with three replicates. Each experimental unit contained 4 plants.

The data were subjected to analysis of variance (ANOVA) using the SAS program (SAS Institute, 2002). The data were statistically analyzed using the methods described by Snedecor and Cochran [33], with L.S.D. at 5% used to compare treatment means.

RESULTS AND DISCUSSION

A. Vegetative growth: Effect of saline water irrigation with seawater dilutions and foliar spraying of seaweed extract on the vegetative growth of *Ruellia simplex*, L. plant.

The results of the biomass characteristics are presented in Tables (3 and 4).

In the first and second seasons, irrigation with diluted seawater at all rates significantly reduced plant growth values compared to tap water (control), as shown in Tables (3 and 4) and Figure (1). The lowest values of plant biomass were obtained with the high rate of seawater irrigation (15%).

All characteristics, including plant height (cm), number of branches per plant, number of leaves per plant, stem diameter (mm), total fresh weight, total dry weight of plants (g), and fresh and dry weight of the roots of the

Table 3: Effect of algae extracts on plant height (cm), no. of branches, no. of leaves per plant and stem diameter (mm) of *Ruellia simplex* L. plants growth under salinity stress during 2021 and 2022 seasons.

Seawater %	Asco. N. extract (ml/l)	Plant height (cm)		No. of Branches/ plant		No. of Leaves/ plant		Stem diameter (mm)	
		1 st	2 nd	1 st	2 nd	1 st	2 nd	1 st	2 nd
0.0%	0 ml	18.55 c	37.43 c	5.44 c	5.33 bc	73.89 c	99.81 c	0.75 a	0.64 a
	2 ml	19.55 b	38.55 b	6.00 b	5.59 b	75.67 b	106.55 b	0.77 a	0.66 a
	4 ml	20.16 a	39.81 a	6.44 a	6.44 a	78.55 a	116.99 a	0.82 a	0.73 a
	x	19.42 a	38.58 a	5.96 a	5.78 a	76.03 a	107.79 a	0.78 a	0.68 a
5.0%	0 ml	17.33 e	35.90 f	4.99 de	4.33 def	67.66 f	88.77 e	0.63 a	0.56 a
	2 ml	17.30 e	36.33 e	5.00 de	4.66 cde	68.44 e	93.22 d	0.65 a	0.60 a
	4 ml	18.05 d	36.81 d	5.33 cd	5.00 bcd	70.77 d	95.94 d	0.70 a	0.61 a
	x	17.56 b	36.35 b	5.11 b	4.66 b	68.96 b	92.64 b	0.66 b	0.59 b
10.0%	0 ml	16.38 f	34.91 h	4.11 g	3.66 fg	59.99 i	80.33 gh	0.57 a	0.50 a
	2 ml	16.60 f	35.00 gh	4.33 fg	4.00 efg	63.44 h	83.70 fg	0.60 a	0.52 a
	4 ml	16.77 f	35.37 g	4.66 ef	4.33 def	65.22 g	86.99 ef	0.61 a	0.54 a
	x	16.59 c	35.09 c	4.37 c	3.99 c	62.88 c	83.68 c	0.59 c	0.52 c
15.0%	0 ml	14.05 i	32.22 k	2.86 i	2.77 h	37.89 l	62.00 j	0.47 a	0.41 a
	2 ml	14.77 h	33.33 j	3.30 h	3.33 gh	43.77 k	75.00 i	0.55 a	0.44 a
	4 ml	15.83 g	34.31 i	3.66 h	3.48 g	58.78 j	78.11 hi	0.65 a	0.49 a
	x	14.88 d	33.29 d	3.28 d	3.19 d	46.81 d	71.70 d	0.53 d	0.44 d
LSD _{0.05%}	Seawater	0.665	0.246	0.2478	0.124	0.771	2.114	0.0155	0.0145
	Asco.	0.576	0.213	0.2146	0.107	0.668	1.8308	0.0135	0.0126
	Sea×Asco.	NS	0.425	NS	0.677	0.422	3.6593	2.699	2.512

Table 4: Effect of algae extracts on the growth of *Ruellia simplex* L. plants under salinity stress during the 2021 and 2022 seasons, in terms of total fresh weight(g), total dry weight (g), fresh root weight (g) and dry root weight (g).

Seawater %	Asco. N. extract (ml/l)	Total fresh weight		Total dry weight (g)		Root Fresh weight (g)		Root dry weight (g)	
		1 st	2 nd	1 st	2 nd	1 st	2 nd	1 st	2 nd
0.0%	0 ml	69.34 bc	64.99 c	19.73 c	19.33 c	38.02 b	34.88	9.38 b	11.99 b
	2 ml	70.47 b	67.44 b	20.96 b	20.11 b	39.53 b	35.89	9.70 b	12.33 b
	4 ml	74.18 a	71.44 a	23.59 a	21.55 a	45.93 a	36.55 a	11.89 a	13.55 a
	x	71.33 a	67.92 a	21.43 a	20.33 a	40.98 a	35.77 a	10.33 a	12.63 a
5.0%	0 ml	63.97 e	56.89 e	17.61 ef	16.33 f	30.56 de	31.44	7.79 cd	11.00 d
	2 ml	66.67 d	59.06 d	18.48 de	17.11 e	32.75 cd	31.89	8.05 c	11.00 d
	4 ml	68.17 cd	59.84 d	19.33 cd	18.66 d	34.98 c	32.99	9.06 b	11.55 c
	x	66.27 b	58.60 b	18.47 b	17.37 b	32.76 b	32.11 b	8.30 b	11.18 b
10.0%	0 ml	54.64 h	50.89 g	15.95 gh	13.77 i	26.14 fg	29.00	6.33 fg	9.55 f
	2 ml	56.54 g	52.89 f	16.36 gh	14.29 h	28.53 ef	29.70	6.92 ef	10.11 e
	4 ml	59.73 f	54.99 e	16.82 fg	15.11 g	29.48 e	30.33	7.33 de	10.66 d
	x	56.97 c	52.92 c	16.37 c	14.39 c	28.05 c	29.68 c	6.86 c	10.11 c
15.0%	0 ml	46.76 j	39.67 j	11.71 j	12.00 l	19.81 h	23.11	4.57 i	7.99 h
	2 ml	49.68 i	45.78 i	14.08 i	12.44 k	21.98 h	25.55	5.52 h	8.33 h
	4 ml	50.94 i	48.66 h	15.25 h	13.33 j	24.48 g	27.55	5.92 gh	8.77 g
	x	49.12 d	44.70 d	13.68 d	12.59 d	22.09 d	25.40 d	5.34 d	8.36 d
LSD _{0.05%}	Seawater	0.888	1.129	0.667	0.2314	1.414	0.269	0.384	0.232
	Asco. N.	0.769	0.978	0.578	0.2004	1.224	0.2326	0.333	0.2006
	Sea×Asco.	NS	1.953	1.156	0.4007	NS	0.463	0.656	0.4007

In a column, means followed by a common letter (s) are not significantly different by LSD at the 5% level.

plants (g), were greatly improved by using algae extract at a concentration of 4 ml.

Table (3) The application of seaweed extracts accelerated the efficient absorption of nutrients, and thus improved the vegetative growth characteristics. Various parameters showed significant differences. At the end of our study, the treatment (4g ANE) had the tallest plants (20.16 - 39.81 cm), in both seasons, respectively.

On the other hand, the significantly, shortest plants were recorded as a result of irrigation by 15% seawater; as gave 14.05 and 32.22 cm in both seasons, respectively.

The highest average maximum number of branches (6.44-6.44), number of leaves (78.55 per plant), and diameter of stems (mm) (0.82-0.73). In the same table, the application of high doses of algae extract (4 ml/l) combined with high saline water irrigation (15%) gave the

Table 5: Effect of algae extracts on the number of flowers per plant, Flower fresh weight (g), Flower dry weight (g) and days A to Z(days) growth of *Ruellia simplex* L. plants under salinity stress during 2021 and 2022 seasons.

Seawater %	Asco. N. extract (ml/l)	Number flowers/plant		Flower fresh weight (g)		Flower dry weight (g)		Days A to Z(days)	
		1 st	2 nd	1 st	2 nd	1 st	2 nd	1 st	2 nd
0.0%	0 ml	6.67 a	4.11 c	0.70 a	0.315 a	0.200 a	0.054 a	70.83 g	66.08 i
	2 ml	5.55 b	5.11 b	0.40 b	0.342 a	0.080 a	0.059 a	65.42 h	75.42 h
	4 ml	4.00 c	7.00 a	0.34 c	0.413 a	0.053 a	0.200 a	56.08 i	80.83 g
	x	5.41a	5.41 a	0.48 a	0.360 a	0.110 a	0.104 a	64.11 d	74.11 d
5.0%	0 ml	3.56 d	3.33 de	0.32 c	0.244 a	0.049 a	0.045 a	77.75 de	84.00 f
	2 ml	3.22 e	3.50 cde	0.31 cd	0.268 a	0.046 a	0.047 a	76.25 ef	86.25 ef
	4 ml	2.56 f	3.67 cd	0.29 cde	0.288 a	0.044 a	0.050 a	74.00 f	87.75 de
	x	3.11b	3.50 b	0.31 b	0.270 b	0.050 b	0.047 b	76.00 c	86.00 c
10.0%	0 ml	2.22 g	1.99 g	0.26 de	0.179 a	0.039 a	0.033 a	85.25 bc	89.50 d
	2 ml	1.88 h	2.56 fg	0.25 ef	0.200 a	0.035 a	0.036 a	82.50 c	92.50 c
	4 ml	1.44 i	2.89 ef	0.20 fg	0.232 a	0.032 a	0.042 a	79.50 d	95.25 bc
	x	1.86c	2.48 c	0.24 c	0.210 c	0.040 c	0.037 c	82.15 b	92.42 b
15.0%	0 ml	1.00 j	0.77 h	0.18 g	0.133 a	0.027 a	0.021 a	93.42 a	96.66 b
	2 ml	1.00 j	1.00 h	0.16 g	0.145 a	0.024 a	0.024 a	87.83 b	97.83 b
	4 ml	1.00 j	1.00 h	0.10 h	0.145 a	0.022 a	0.028 a	86.67 b	103.42 a
	x	1.00 d	0.93 d	0.15 d	0.140 d	0.024 d	0.024 d	89.31 a	99.31 a
LSD _{0.05%}	Seawater	0.147	0.331	0.037	0.0095	0.007	0.001	1.677	1.678
	Asco.	0.127	0.2869	0.032	0.0082	0.006	0.0008	1.453	1.4531
	Sea× Asco.	0.251	0.6425	0.054	5.208	3.850	5.293	2.903	2.903

In a column, means followed by a common letter (s) are not significantly different by LSD at the 5% level.

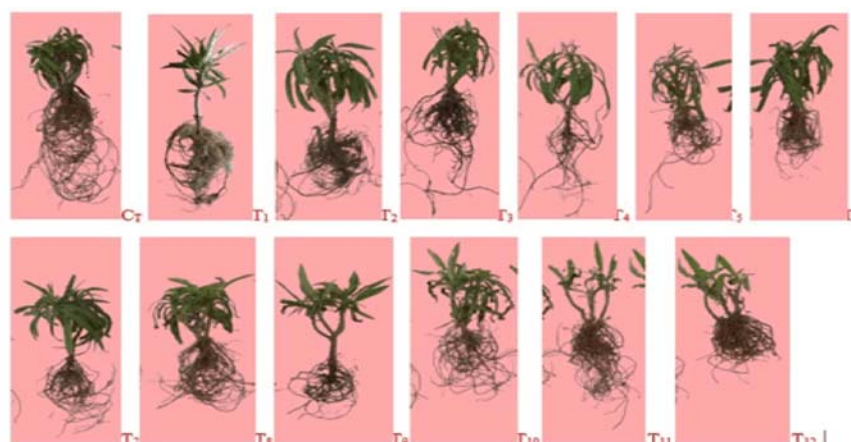


Fig. 1: Effect of treatment with algae extracts during vegetative phase of *Ruellia Simplex* plant grown under salinity stress. [CT=control, T1=0 SW.& 0Asc. Extr., T2=0 SW.&2ml Asc. Extr., T3= 0SW&4ml Asc. Extr., T4=5%SW.&0Asc. Extr., T5=5% SW.&2ml Asc. Extr., T6=5% SW.&4 ml Asc. Extr., T7= 10% SW.& 0 ml Asc. Extr., T8= 10% SW.&2ml Asc. Extr., T9=10% SW.&4 ml Asc. Extr., T10=15% SW.&0 ml Asc. Extr., T11=15% SW.&2ml Asc. Extr., T12= 15% SW.&4ml Asc. Extr.].

lowest number of leaves per plant (58.78 & 78.11) and the lowest diameter of stems (0.65 & 0.49 mm) compared to the control in both seasons, respectively.

The results in Table (4) showed that increasing the applied concentration of algae extract (4 ml) increased the total fresh weight, total dry weight, root fresh weight and root dry weight in all the applied methods. However, the application of 15.0% seawater resulted in lower values of total fresh weight (46.76 g), total dry weight (11.71 g), root fresh weight (19.81 g) and

root dry weight (4.57 g). The same trend was observed in the second growing season with slightly higher or lower values.

B. Flowering growth: The results presented in Table (5) showed that the effect of algae extract applied with seawater rates application had significant effects on the number of flowers/ plants, fresh flower weight (g), dry flower weight (g) and days A to Z (days) during the growing seasons 2021 and 2022.

Table 6: Effect of algae extracts on N, P, K, Ca and Na percentage in leaves of *Ruellia simplex* L. plants under salinity stress during the 2021 and 2022 seasons.

Seawater %	Asco. N. extract (ml/l)	N %		P %		K %		Ca %		Na %	
		1 st	2 nd	1 st	2 nd	1 st	2 nd	1 st	2 nd	1 st	2 nd
0.0%	0.0ml	10.40c	11.10c	3.50bc	4.10c	40.80c	41.90bc	24.53c	25.46bc	26.001j	265.00f
	2.0ml	10.63b	11.33b	3.56b	4.26b	43.33b	42.70cd	25.43b	26.13b	26.001j	241.66g
	4.0ml	12.00a	12.90a	3.70a	4.40a	44.03a	46.33a	25.56a	28.43a	174.00k	180.33h
	Mean	11.01	11.77	3.58	4.25	42.72	43.64	25.17	26.67	232.00	228.99
5.0%	0.0ml	8.33f	9.26f	3.40de	3.90d	40.53e	41.40cd	24.23e	24.9bcd	282.00g	275.33e
	2.0ml	8.86e	9.73e	3.46cd	3.90d	40.60de	41.53cd	24.33d	25.00bc	268.33h	269.33ef
	4.0ml	9.13d	9.90d	3.50bc	3.90d	40.66d	41.70cd	24.46c	25.13bc	262.33i	266.66ef
	Mean	8.77	9.63	3.45	3.90	40.60	41.54	24.34	25.01	270.88	270.44
10.0%	0.0ml	7.73h	8.16h	3.30f	3.70e	39.83h	39.36e	22.90h	23.4def	308.33d	314.66c
	2.0ml	7.80gh	8.23h	3.33ef	3.76e	40.26g	40.93d	23.10g	24.1cde	304.00e	310.33c
	4.0ml	7.93g	8.50g	3.40de	3.86d	40.43f	41.26cd	24.03f	24.7bcd	292.66f	290.33d
	Mean	7.82	8.3	3.34	3.77	40.17	40.52	23.34	24.05	301.66	305.10
15.0%	0.0ml	7.26j	7.80j	2.80h	3.16g	34.40k	34.86g	21.66k	22.40f	352.66a	358.66a
	2.0ml	7.53i	7.86j	3.20g	3.53f	35.83j	35.43g	22.43j	23.23ef	343.66b	346.66b
	4.0ml	7.70h	8.03i	3.26	3.60f	37.86i	36.93f	22.56i	23.30ef	310.00c	318.00c
	Mean	7.50	7.90	3.08	3.43	36.03	35.74	22.22	22.97	335.44	341.10
LSD _{0.05%}	Seawater	0.085	0.039	0.041	0.051	0.054	0.048	0.049	0.092	0.510	0.520
	Asco.	0.074	0.034	0.035	0.044	0.047	0.042	0.042	0.079	0.442	4.510
	Sea× Asco.	0.14	0.067	0.069	0.089	0.094	0.839	0.085	1.590	0.880	9.020

Table 7: Effect of algae extracts on chlorophyll, proline, carbohydrate % and MSI% in leaves of *Ruellia simplex* L. plants under salinity stress during the 2021 and 2022 seasons.

Seawater %	Asco. N. Extract (ml/l)	Chlorophyll (SPAD)		Proline (mg/g)		Carbohydrate (mg/100g)		MSI %	
		1 st	2 nd	1 st	2 nd	1 st	2 nd	1 st	2 nd
0.0%	0.0ml	38.86b	43.63	0.69a	0.73a	57.96c	60.23c	62.33bc	65.33c
	2.0ml	39.36b	44.16	0.70a	0.71a	63.00b	64.13b	63.00b	66.66b
	4.0ml	40.76a	45.70	0.66a	0.66a	69.00a	69.83a	64.66a	69.00a
	Mean	39.66	44.5	0.68	0.70	63.32	64.73	63.33	67.00
5.0%	0.0ml	35.50e	40.43	0.89a	0.91a	41.93f	42.83f	61.0ef	63.00e
	2.0ml	36.73d	41.03	0.86a	0.89a	42.13e	43.20e	61.33de	64.00d
	4.0ml	37.83c	41.46	0.75a	0.75a	48.90d	49.93d	62.00cd	65.00c
	Mean	36.68	40.97	0.83	0.85	44.32	45.32	61.44	64.00
10.0%	0.0ml	29.70h	38.50	1.04a	1.08a	28.13i	30.13i	58.66gh	61.00f
	2.0ml	30.66g	39.03	1.00a	1.05a	37.96h	39.63h	59.00g	61.33f
	4.0ml	32.26f	39.46	0.94a	0.95a	38.90g	40.10g	60.33f	62.66e
	Mean	30.87	39.0	0.99	1.02	35.00	36.62	59.33	61.66
15.0%	0.0ml	21.63k	31.60	1.33a	1.35a	17.40l	18.03l	56.33j	57.33h
	2.0ml	26.23j	34.56	1.24a	1.31a	25.03k	26.46k	57.66i	60.00g
	4.0ml	27.63i	36.56	1.17a	1.17a	26.03j	26.90j	58.00hi	61.00f
	Mean	25.16	34.24	1.24	1.27	22.82	23.80	57.33	59.44
LSD _{0.05%}	Seawater	0.540	0.520	0.017	0.010	0.059	0.110	0.413	0.430
	Asco.	0.467	0.450	0.014	0.010	0.515	0.090	0.358	0.380
	Sea× Asco.	0.940	0.910	2.970	4.810	0.090	0.190	0.720	0.770

In a column, means followed by a common letter (s) are not significantly different by LSD at the 5% level.

In the first season, the control treatment gave the highest number of flowers/ plant (6.67) followed by 2 ml algae extract (5.55), however, 15.0% seawater with 0, 2 and 4 ml of algae extract application gave the lowest number of flowers/ plant (1.00) in the first season. but in the second season the high value was with 4 ml algae extract and the low value with 15.0% seawater treatments.

C. Chemical composition: The results in Table (6) showed that the concentration of some elements especially N%, P%, K% and Ca%, decreased with increasing salinity, while the concentration of Na increased significantly in the plant extract. The control treatment, which had no seawater (tap water) concentration during irrigation, had significantly the highest concentration of N%, P%, K%

and Ca%, compared to the other treatments, while Na concentration was the higher amount in (15% seawater + 0 ml/l Asco. N. extract) than other treatments.

In addition, the results of Table (7) showed that treatment algae 4.0 ml significantly increased chlorophyll, carbohydrate, and MSI% (40.76, 69.0, and 64.66) respectively in the first season and by (45.70, 69.83, and 69) respectively, in the second season compared to the control. The results of Table (7) show that the treatment with the highest concentration of salinity gave the highest rate of proline in the plants in the 2 seasons; it is the highest value of proline content (1.24 & 1.27) with 15% seawater irrigation. In both seasons, the seaweed extract treatments significantly changed the proline content in the leaves of the plants irrigated with saline water. The 0.4 ml of seaweed *A. nodosum* extract gave the minimum value of proline content (mg/g).

DISCUSSION

Desiccation of arid and semi-arid areas, soil or water salinity is one of the limiting environmental factors affecting the yield of agricultural and medicinal plants, which disrupting the natural growth and development of the plants in large areas of the planet [34]. The study proposed the effect of treatment with algae extracts on growth, flowering and chemical composition of *Ruellia simplex* plant grown under salinity stress. At all salinities, foliar application of seaweed extract showed that Asc. (4.0 g), especially at 15% salinity, had a significant effect on biomass compared to other bio-stimulatory therapies (Table 5). Due to osmotic stress, saline irrigation typically results in a loss of swelling [35]. Asc. mitigated the effects of high salinity by inhibiting meristematic activity, cell elongation, and water uptake, thereby reducing relative water content (RWC). Accumulation of salts in the root zone affects plant performance by creating a water deficit and disrupting ion homeostasis [36]. which in turn affects metabolic dynamics functions. These stresses alter the hormonal status and affect basic metabolic processes. The result is growth inhibition and reduced yield [36, 37]. By causing osmotic stress, ionic and nutrient imbalance, and osmotic stress, salinity is a significant abiotic influence that negatively affects plant growth. According to Zhang *et al.* [38], such imbalances negatively affect a variety of physiological and biochemical pathways involved in plant growth and development.

The results of the present study (Table 6) show that salt stress inhibited plant growth by significantly reducing plant height, number of branches and leaves,

stem diameter, total fresh and dry weight, root fresh and dry weight, number of flowers, flower fresh and dry weight with increasing seawater concentration in all treatments. Compared to the other treatments, the growth reduction in seawater 15% + 0.0 algae extract was extremely marked, whereas 0.0% seawater + 4 ml/l algae extract showed a superior adaption. Similar studies carried out on various plants also support our findings [35, 39]. Furthermore, the fresh and dry biomass was strongly reduced in all treatments of algae extract concentrations under all seawater treatments. Expect (Seawater× Asco.) showed no significance for total fresh and root weight in the first season. Previous studies on various plants have shown that the fresh and dry weights of roots and shoots [40-42]. Numerous studies have documented a significant reduction in chlorophyll content under salinity stress [43-45]. The influence of salinity on the chlorophyll content has been studied from different perspectives.

It has been reported (Table 4) that the increase in yield with the application of seaweed extracts (SEs) applications was associated with improved chlorophyll biosynthesis (higher SPAD index) [46]. Our results obtained at the seedling and elongation stages showed that spraying SEs could significantly increase the SPAD value of sugarcane leaves, indicating that the application of seaweed extracts (SEs) increased the chlorophyll content in the leaves (Table 6), which is also supported by other reports [47-49]. This could be due to the presence of betaine, amino acids, and other active ingredients in seaweed extracts (SEs) that inhibit chlorophyll degradation [50]. Seaweed extracts also contain magnesium, which is required for chlorophyll synthesis [51]. Our results showed that spraying seaweed extracts (SEs) had a significant effect on the photosynthetic rate of sugarcane leaves, consistent with that of SPAD, and this resulted in a greater ability of the plants to maintain a better photosynthetic performance [52, 53].

The reduction in leaf chlorophyll content under NaCl stress has been attributed to the destruction of chlorophyll pigments and the instability of the pigment-protein complex. It is also attributed to the interference of salt ions with the de novo synthesis of proteins, the structural component of chlorophyll, rather than to the degradation of chlorophyll. Thus, soil salinity has been shown to negatively affect the growth and photosynthetic metabolism of *Catharanthus roseus* (L.) [54].

Nutrients present in the seaweed extracts are readily absorbed by leaves through stomata and hydrophilic

pores in the cuticle. Foliar application of seaweed extract products, including those of a commercial *A. Nodosum* extract increased the Cu uptake in grapevine probably through increased permeability of the cell membrane. In another study, the application of a commercial extract of *E. maxima* to lettuce grown under optimum conditions was found to improve yield and leaf Ca, K and Mg concentrations [55].

Changes in leaf growth under saline stress are associated with leaf Na⁺ content in Triticum and soybean [56, 57]. This is shown in the results (Table 7).

Similarly, higher NaCl salinity resulted in higher leaf Na⁺ concentrations in Cape gooseberries [58].

Our results in Table 4 are consistent with what was mentioned by Ali *et al.* [59], that seaweed extracts contain nutrients necessary for plants, as they contain the major nutrients K, P, N and the minor nutrients Fe, B, Mg, Zn, Mo, Cu, as well as plant hormones such as auxins, gibberellins, cytokinin, and these hormones, when added to the soil or sprayed on plants, stimulate root growth, increase stem thickness, and increase vegetative growth by increasing the efficiency of photosynthesis. These amino acids help plants withstand harsh conditions such as cold, salinity, drought, and heat. It has played a role in the synthesis of chlorophyll and stimulates root formation and cell division.

One of the main ways in which higher plants adapt to salt stress is through the accumulation of appropriate solutes, such as proline, a preferred organic osmoticum in many plant species [60]. Osmotic correction is achieved through the use of compatible solutes, and to maintain the functional state of macromolecules, most likely by scavenging ROS [61]. According to Türkan and Demiral, [62], salt-tolerant plants have higher proline accumulation in their leaves than salt-sensitive plants.

Another physiological measure that is frequently used to assess the potential salinity tolerance of plant species is MSI, which is considered to be a sign of stress tolerance [63-66]. In addition, according to Farooq and Azam, [67], MSI is a useful physiological trait for screening resistant genotypes at the seedling stage. According to Ahmed *et al.* [68], there is a direct correlation between MSI and reactive oxygen species (ROSs) induced lipid peroxidation, which generates malondialdehyde (MDA), during salinity stress. Furthermore, this is a quantitative trait with a strong genetic correlation with grain yield through moderate heritability [69-72]. According to our results (Table 7), plants exposed to salt stress showed a slight change in their MSI when compared to control conditions. The reason for the superiority of the seaweed extract

treatments is due to the effect of nutrients on photosynthesis, respiration and cellular metabolism, as they are involved in the synthesis of nucleic acids necessary for cell division and the formation of proteins, enzymes and hormones, especially nitrogen, which leads to increased plant branching and vegetative growth [73].

CONCLUSION

In short, applications of the *Ascophyllum nodosum* extract significantly increased yield and flower quality, similar to those shown in previous experiments and field trials. The researchers looked at how the *Ruellia* plants grew, the quality of their flowers, and the chemicals they contained. They found that spraying 4.0 ml of the best seaweed extract (*A. nodosum*) and watering them with tap water (control) significantly affected these factors. Furthermore, the data suggest that foliar spraying with spray (ANx) in the range of 2 to 4 ml is effective. Finally, the results suggest that seaweed extract could improve plant growth and quality in saline environments. Saline water concentrations of 5% and 10% were suitable for marketing the plant, and enabled it to achieve its objective.

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