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Mitigation Negative Effects of Salt Stress on Common Bean (*Phaseolus vulgaris*) Using Seaweed Extracts

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Abstract

The abiotic stress can significantly affect plant growth and its biochemical traits, consequently the economic yield of crops. Therefore, our study aimed to investigate the effects of seaweed extract application (i.e. 0, 100 and 300 ppm red algae extracts) on growth, biochemical and tolerance traits of salt-sensitive *Phaseolus vulgaris* seedling grown under saline stress conditions (50 and 100 mM NaCl). Plant growth traits and chlorophyll contents were significantly decreased when plants were grown with saline treatments. However, seaweed treatments either 100 ppm or 300 ppm mitigated the salt stress and significantly improved the total phenolic, proline, pigments contents and enzymes activity. Saline stress resulted in an increase in the electrolyte leakage (EL), while the seaweed extraction treatments minimized EL and malondialdehyde (MDA) contents. In addition, proline content and antioxidant enzymes activity were significantly increased in the response to salt stress treatments. Compared to the 300 ppm of the algae extract, it was clear that the red algae extract at 100 ppm was the optimal treatment in terms of improving plant growth and biochemical traits when plants grown under the highest level of saline stress (100 mM NaCl). In conclusion, treating seeds of crops with the algae extracts can significantly mitigate the harmful effects of saline stress.

Keywords: *Phaseolus vulgaris*, Saline stress, Seaweed extract, Red algae, Biochemical traits, Enzymes activity.

1. Introduction

Salinity is one of the most major environmental problems that can affect directly or indirectly agricultural crops production. Many crops are sensitive to saline stress and unable to tolerate even the low levels of salinity [1].Meanwhile, saline stress is considered one of the most major abiotic stresses limiting the growth and productivity of common beans in arid and semiarid regions under field conditions [2].In the arid and semiarid areas, salinity could be caused by the poor irrigation water that can contain considerable amounts of salts, accumulation of salts in the top layer of the soil due to over-irrigation, and proximity to the sea as well as the capillarity rise of salts from underground water into the root zones because of the excessive evaporation. Besides the low precipitation, high evaporation rate, and poor water management could cause salinity-related problems in these regions [3].Even moderate degrees of salt stress can cause a reduction of 50–70% in the economic yields for most crops compared to the yield obtained from crops grown in standards conditions [4, 5].Therefore, there are more than 800 million hectares of arable land potential for agricultural use, severely affected by the salinity and has become unproductive [6].

Elevated soil salinity has several deleterious effects manifested upon plants including inactivation of enzymes, inhibition of protein synthesis, premature leaf senescence, decreased rate of photosynthesis and respiration as well as loss of cellular integrity. Furthermore, high salt stress can decrease the accessibility of K^+, Ca^+ and increase the accumulation of Na^+, Cl^- which can cause an ionic imbalance resulting in a reduction for the nutrient uptake by the plants [7].Moreover, saline stress can induce alterations in plant metabolism and accumulation of reactive oxygen species (ROS) that can have damage

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effects on the proteins, lipids, and nucleic acids [8, 9]. In plants, ROS production under stress is mainly attributed to the photorespiration increment, β-oxidation of fatty acids, and activity of the mitochondrial electron transport chain [10]. On the other trend, saline stress can induce the defense systems by enhancing antioxidant activities to minimize the damaging effects of the free radicals [11].

Plants can posse various mechanisms for the resistance of salinity; therefore, the administration of the external plant bio-stimulators may ameliorate salinization drawbacks [12]. The most commonly used bio-stimulants are based on the humic compounds, amino acids, seaweed extracts, and microalgae [13-15]. Different studies reported that algae extracts can stimulate plant growth, enhance photosynthetic activity, increase the resistance to plant diseases, and tolerance to the adverse environmental conditions, thereby improving the yield and productivity of crops [16-19]. This is due to the seaweed-based bio-stimulants, which contain cytokinins, auxins and other hormone substances [20].Seed priming with algae extracts is a promising alternative application to stimulate growth, development, and mitigate the harmful effects of salinity on plants [21]. Thus, according to the increasing demand for organic food as well as growing environmental awareness, the bio-stimulants market is rabidly upward all over the world [22].Consequently, the bioactive substances extracted from marine algae are used in modern agricultural and horticultural crops as bio-fertilizers, bio-stimulants, or bio-regulators to improve crop quality and yield of economically important crops [23].

Common bean (*Phaseolus vulgaris* L.) is considered one of the most important vegetable crops grown worldwide. Common bean is the most consumed legume by human particularly in developing countries since it an essential component of the diet as a source of proteins, vitamins, minerals, and fibers. Bean seeds contain approximately 90% dry matter, including 60% carbohydrates (mainly starch 40% and dietary fiber 16%), 22% protein, and mineral salts [24, 25].In Egypt, beans are widely cultivated on the newly reclaimed soils. However, most of these soils are affected by different levels of salinity [26].Common beans and other legumes are regarded as suitable crops for the enhancement of bio-productivity and the reclamation of the marginal lands because they can be used for food and fodder as well as can enrich the soil nitrogen in symbiotic association with rhizobium [27]. Therefore, they can significantly improve soil fertility in the tropical and subtropical zones where most of these soils are salinized [28].However, common bean is sensitive to the salt stress compared to other crops such as barley (*Hordeum vulgare* L.) or cowpea (*Vigna unguiculata* L.),[29, 30].

Therefore, our study aimed to investigate the effects of seaweed extract application (i.e. 0, 100 ppm and 300 ppm red algae extracts) on growth, biochemical and tolerance traits of salt-sensitive *Phaseolus vulgaris* seedling grown under saline stress (50 and100 mM NaCl).

2. Materials and Methods

Seaweed collection and preparation of extracts

The seaweed, *Gelidiumvagum* (Fam. Rhodophyceae) was collected from Abu-Qir Beach, Coastal region of Alexandria, Egypt. The seaweed was identified in the National Institute of Oceanography and Fisheries (NIOF), Alexandria Morphologically distinct thallus of algae was placed separately in the new polyethylene bags and was kept in an icebox containing slush ice and directly transported to the laboratory. The samples were washed thoroughly using distilled water to remove the salt from the surface of the sample. The water was drained off and thallus was spread on blotting paper to remove the excess water. The powdered samples were then extracted with 80% MeOH for 24 h under continuous shake at 20°C. The extract was then concentrated under a vacuum in a rotate-vapor at 40°C. The solid mass obtained was then dissolved in water and the concentration was adjusted to 100 ppm and 300ppm [31].

Plant material and treatments

Green bean seeds (*Phaseolus vulgaris*, L. cv. Paulista,) were used in this study as a sensitive plant to the salt stress. Initially, the seeds were divided into 3 groups; the first group was soaked in distilled water as a control. The second and third group seeds were

treated by soaking in 100 ppm and 300 ppm red algae extracts respectively. Then, treated seeds were sown in polyethylene bags (4×8×13 cm) containing 700 g acid-washed sandy soil (2 seeds/bag). The seeds were irrigated with the nutrient solution that was prepared according to [32] and [33].

For15 days after planting, the irrigation with Hoagland nutrient solution was applied in the first group as a control, while the nutrient solution containing 100 and 300 ppm red algae extracts were used in the second and third groups, respectively. The irrigation was carried out daily using the nutrient solution twice, while the third time of irrigation was carried out using distilled water to avoid the nutrient accumulation.

After 15 days, each group was divided into 3 subgroups. Plants (30-days old) were treated with salinity (50mM NaCl) in the first subgroup; while they were treated with (100mM NaCl) in the second subgroup, and did not treat and used as a non-stressed treatment in the third subgroup. After 7 days of treatment with NaCl, seedlings (37-days old) were collected and stored at -20°C for the further biochemical analysis.

Measurements

Leaf area and water content:

Leaf area (LA) were determined According to [34] using a model for LA estimation of snap bean. Trifoliate leaf is (LA = 1.5198 LtWt) as the length of the leaflet (Lt) and width of the leaflet (Wt)

Dry weight was determined according to **[35]**. A known weight of leaves was dried in a ventilated oven at 70°C for reaching the constant weight. Plant dry weight was determined and expressed as g.

Leaf water content % = (f.w-d.w)/ (f.w) ×100

Biochemical analysis:

Total phenols

Total soluble phenolic concentration was determined in ethanolic extract 80% according to **[36]** by the Folin-Ciocalteu method using the standard curve of catechol. A known fresh weight of the leaf tissue was immersed in ethanol 80%, and was kept in a dark bottle for 24 h at 0˚ C. The samples were re-extracted three times, then the extract was collected. Then, 0.5 mL of the extract was placed into test tubes; 2.5 mL of Folin-Ciocalteu's reagent and 2 mL of sodium carbonate (7.5%) were added. The tubes were mixed and allowed to stand for 30 min. The absorbance was recorded at 650 nm using a spectrophotometer (UV-Vis spectrophotometer UV 9100 B, LabTech).

Proline

Proline content was analysed using a ninhydrin colorimetric method of **[37]** as modified by **[38]**. Frozen tissues of plant leaves were ground using mortar and pestle and homogenized with 100 mM sodium phosphate buffer (pH=6). The extraction ratio was 10 mL for each g of plant tissue. After that, the homogenate was centrifuged for 10 min at 4500 rpm, then 200 µLof the extract was reacted with 1 mL of ninhydrin solution (2.5 g dissolved in 100 mL of orthophosphoric acid, acetic acid, and water 15:60:25, V:V:V) for 1 h in boiling water. Thereafter, the developed dye was extracted with 1 mL toluene and vigorously vortexes for 15 seconds. The toluene phase was sucked using a micropipette and the absorbance was measured at 515 nm using a spectrophotometer (UV-Vis spectrophotometer UV 9100 B, LabTech).

Total Carbohydrate content

Total carbohydrate was extracted according to **[39].** An air-dried sample of0.1 g was hydrolysed with 1N HCl by refluxing 6h in boiling water bath. The resulting solution was filtered, neutralized and the total volume was made up to 100 mL with distilled water. The total carbohydrates were determined calorimetrically 1 mL of the sample by alkaline potassium ferricyanide reagent.

Reducing Sugars

Reducing sugar content was analysed by dinitrosalicylic (DNS) colorimetric method of [40], using d-glucose as a standard. For each of the 0.2 mL of the leaf ethanolic extract sample, 3 mL of DNS reagent and 2.8 mL deionized water were added. The mixture was then heated in boiling water for 5 min until the red-brown colour was developed. Then, the mixture was cooled in the room temperature. The absorbance was then measured with a spectrophotometer at 540 nm. The concentration of total reducing sugars was calculated based on a standard curve obtained with glucose.

Free Amino acids

In the ethanol extract, Free amino acids were determined with ninhydrinreagent according to **[41]**.Ninhydrin solution was prepared by dissolving 2.0 g ninhydrin in 25 mLof acetone followed by the addition of 25 mL0.2 M acetate buffer (pH 5.5).Added 1 mL of ninhydrin solution with 1 mLsample extract and 3 mldistilled water in the same tube, mixed well and cooled in a boiling water bath for 15 min. Optical density was measured at 570 nm using a spectrophotometer and free amino acids content was calculated as glycine from the standard curve.

Plant pigments

For the chlorophylls and carotenoids analyses, 0.2g of fresh leaves were extracted by grinding the tissue with a mortar and pestle using 10 mL N, N dimethylformamide **[42].** The resulting extracts were incubated in the dark fridge overnight. The total chlorophyll and carotenoids concentrations were measured using a spectrophotometer. The absorbance of the solution was recorded at 470, 647 and 664 nm. The concentration of the total chlorophyll and carotenoids was calculated as follows:

$$Chlorophyllaconc. = (12\ A_{664}) - (3.11\ A_{647})$$
$$Chlorophyllbconc. = (20.78A_{647}) - (4.88\ A_{664})$$
$$Carotenoids\ conc. = ((1000\ A_{470}) - (1.12\ Chl.a) - (34.07\ Chl.b))/245$$

Malondialhyde (MDA)

MDA was determined according to the method of [43]. The level of lipid peroxidation was determined in terms of (MDA) by using the thiobarbituric acid (TBA) test. Frozen tissues were homogenized in 0.1% (w/v) trichloroacetic acid (TCA). The extraction ratio was 10 mL for each gram of plant tissue. The homogenate was centrifuged at 5000 rpm for 10 min. The reaction mixture contained 1 mL from the supernatant and 4 mL 0.5% (w/v) TBA were dissolved in 20% (w/v) TCA. The mixture was heated in boiling water for 30 min then the mixture was cooled at room temperature and centrifuged at 5000 rpm for 15 min. The absorbance of the supernatant was measured at 535 nm using a spectrophotometer.

Electrolyte leakage (EL %):

The plant cell membrane permeability due to salinity stress was assessed through electrolyte leakage measurement using an electrical conductivity meter and calculated as per [44].Sharp leaf discs were made and transferred to a test tube containing 5 mL of distilled water. The tubes were shake for 4 h at room temperature then measure the conductivity in the solution (reading1).Solution containing the leaf discs were autoclaved. After the liquid cools down, the conductivity of the solution were measured again (total ions present in the leaf discs). Ion leakage was represented as the percentage of total ions released (Reading1/Reading2 × 100).

Antioxidants activities

Fresh green leaves (500 mg) were ground to a fine powder in liquid N and extracted with 5 mL of chilled extraction buffer including 50 mM K-phosphate buffer, pH 7.6, and 0.1 mMNa2-EDTA. The mixture was centrifuged at 20,000g for 30 min at 4°C. The supernatant (enzyme extract) was used to measure various antioxidant enzyme activities following the method of [45]. Soluble proteins concentration was quantified in the crude enzyme extract by the method of **[46]** using bovine serum albumin as a standard.

Peroxidase activity (POD)

Peroxidase activity was quantified by the method of **[47].**The assay mixture (100 mL) contained 10 mL of 1% (v/v) guaiacol, 10 mL of 0.3 % H_2O_2, and 80 mL of 50mM phosphate buffer (pH=6.6). The absorbance was recorded every 30 second for 3 min on a spectrophotometer at 470 nm.

Catalase activity (CAT)

Catalase (CAT) activity was determined according to the method of **[48]** as modified by **[45]**. CAT activity was measured by monitoring the decrease in absorbance at 240 nm following the decomposition of H_2O_2 for 1 min using a spectrophotometer.

Superoxide dismutase (SOD)

Superoxide dismutase (SOD) assay was based on the method described by [49]. The activity of SOD was determined by inhibiting the photochemical decrease of nitro blue tetrazolium (NBT) at 560 nm. One unit of SOD activity was characterized as the sum of the enzyme that inhibited 50% of NBT photo reduction.

Ascorbate peroxidase (APX)

Ascorbate peroxidase (APX) activity was measured according to the method of **[50]**by determining the decline in the assimilation of the oxidized ascorbate at 290 nm. One unit of APX was defined as the amount of enzyme required to consume 1 µmol ascorbic acid min^{-1}.

Phenylalanine ammonia-lyase (PAL)

Phenylalanine ammonia-lyase (PAL) activity was quantified by the method of **[51].** The reaction mixture consisted of 100 µL crude enzyme, 1.9 mL of 0.05 M Tris-HCl buffer (pH 8.8), and 1 mL of 20 mM L-phenylalanine. The reaction was allowed to proceed for 1h at 37ºC, then the reaction was terminated by the addition of 0.2 mL of 6 M HCl. One unit of enzyme activity was defined as the amount of enzyme that caused an increase in absorbance of 0.01 per hat 290 nm.

Statistical analysis

Data were statistically analysed using Costas software (version 6.4, CoHort Software, USA) according to the method described by **Gomez and**

Gomez [52]. All data were subjected to a two-way analysis of variance and the means were compared by Duncan's multiple range test at a significant level $P \leq 0.05$ **[53].**

3. Results

The growth parameters such as leaf length, leaf width, leaf area index, and leaf fresh weight as well as and moisture content of common bean plants treated with seawed extracts (i.e. 0, 100 ppm and 300 ppm red algae extracts) and gown under saline stress (50 and100 mM NaCl)are represented in **Table (1)**. Different plant growth traits were significantly reduced by increasing the salt stress levels compared to the control treatment, which reflected the NaCl toxicity in the highest level (100 mM NaCl). Meanwhile, using algae extracts (i.e. 0, 100 ppm and 300 ppm) significantly promoted plant growth traits and moisture content of common beans grown under salt stress. In addition, it could be noticed that different plant growth characteristics were not significantly differed when the red algae extracts were applied either at 100 or300 ppm.

Table 1: Effects of red algae extracts application on common bean growth traits and moisture content grown under salt stress.

NaCL(mM)		Leaf length (cm)	Leaf width(cm)	Leaf area (cm^2)	Leaf FW (g)	Moisture (%)
0		2.81 a	2.44 a	10.55 a	2.50 a	86.14 a
50		2.21 b	1.90 b	6.41 b	2.43 a	85.61 a
100		2.04 c	1.71 c	5.35 c	2.64 a	86.48 a
LSD 0.05		0.087	0.136	0.527	0.335	1.236
Red Algae (ppm)						
0		2.11 b	1.78 b	5.81 b	2.53 a	86.74 a
100		2.48 a	2.11 a	8.06 a	2.54 a	86.23 ab
300		2.48 a	2.17 a	8.44 a	2.5 a	85.27 b
LSD 0.05		0.08734367	0.13611528	0.52741995	0.33562867	1.23647722
Interaction Effects						
0	0	2.47 c	2.13 c	8.00 c	2.35 a	87.07 abc
	100	2.83 b	2.47 b	10.62 b	2.56 a	85.81 abc
	300	3.13 a	2.73 a	13.02 a	2.58 a	85.55 bc
50	0	2.07 f	1.63 ef	5.13 gh	2.63 a	87.37 ab
	100	2.37 cd	2.03 cd	7.32 cd	2.34 a	84.80 c
	300	2.20 ef	2.03 cd	6.79 de	2.33 a	84.67 c
100	0	1.80 g	1.57 f	4.29 h	2.61 a	85.78 abc
	100	2.23 de	1.83 de	6.24 ef	2.72 a	88.07 a
	300	2.10 ef	1.73 ef	5.52 fg	2.59 a	85.58 bc
LSD 0.05		0.087	0.136	0.527	0.335	1.236

The same letters in each column indicate no significant differences among different treatments according to the least significant difference LSD test ($P \leq 0.05$).

The total phenolic, proline, reduced sugars, total carbohydrates, and free amino acids contents were significantly differed under different treatments of salt stress and red algae extracts (Table 2). The highest values of proline, total phenolic compounds, carbohydrates, free amino acids, and reduced sugars contents were obtained from plants grown with the highest salt stress levels NaCl (100mM)and algae extract application of 100 ppm followed by plants grown with50 mM NaCl and algae extract of 300 ppm (Table 2). Despite the high concentration of red algae extract at 300 ppm combined with high saline stress at 100 mM NaCl could lead to a decrease in all components when compared to other treatments with red algae.

From data presented in **Table 3**, the photosynthetic pigments such as chlorophyll A and B contents of common bean leaves were significantly decreased by increasing levels of salt stress. However, the application of algae extracts mitigated the undesirable effects of salt stress and enhanced the plant chlorophyll traits. It significantly increased total chlorophyll A and B contents whereas decreased carotenoids at when plants grown under salt stress. On the other hand, the MDA contents and El% were significantly increased in common bean plants by increasing salt stress (Table 3). Nevertheless, the

exogenous application of red algae extracts reduced the negative effects of salt stress treatments and significantly decreased malondialhyde content and electrolyte leakage in common bean plants. Concerning the interaction effects between salt stress treatments and red algae extracts, it was noticed thatthe contents of malondialhyde and electrolyte leakage were significantly decreased in plants stressed with50 or100 mM NaCl in combination with100 or 300 ppm of algae extracts application. In conclusion, the application of red algae extract at 300 ppm is considered the optimal level compared to other treatment (i.e. 100 ppm).

Concerning the activities of peroxidase (POD), superoxide dismutase (SOD), ascorbate peroxidase (APX), catalase (CAT) and phenylalanine ammonia-lyase (PAL) enzymes, data revealed that they were significantly boosted by the increasing of salt stress levels and algae extracts application at the same time. Application of red algae extracts significantly increased all antioxidant enzymesin plants grown under the level of 50 mM salt stress, while, the highest level of salt stress (100 mM) resulted in a reduction in the antioxidant enzymes, especially SOD, CAT, and PAL combined with the level of 300 ppm algae extract (Table 4). The increments in the antioxidant enzymes can support the plants to overcome the negative effects of salt stress. In addition, it is clear that the level of 300 ppm algae extract is the optimal concentration of red algae extracts when compared to the other treatments; although, the use of red algae extracts at the level of 100 ppm showed higher enzymes activities but only when plants grown in soil treated with 100 mM NaCl.

4. Discussion

Salinity stress can possess an adverse effects for the yield, root and leaf traits, water relations, ion uptake, and photosynthesis of the different crops [1, 3, 54]. This can be due to the inhibiting of plant metabolism when grown under salt stress [55].Thus, the usage of red algae extracts could enhance plant growth and improve the crop productivity grown under different levels of salt stress. This is due to the presence of the bioactive substances such as macro and microelement nutrients, amino acids, and growth hormones, which stimulate the cellular metabolism in treated plants leading to enhance plant growth and increase the productivity [23, 56].

Table 2: Effects of red algae extracts on total phenolic compounds, proline, reduced sugars, total carbohydrates, and free amino acids contents of common bean plants grown under salt stress.

NaCLmM	Phenolic (µg/g F.W)	Proline (µg/g F.W)	RS (µM/g F.W)	Carb. (µM/g D.W)	FAA (mg/F.W)
0	102.48 c	5.55 c	166.4 c	262.58 c	9.03 b
50	158 b	8.16 b	275.63 b	310.54 a	11.81 a
100	172.82 a	9.61 a	323.97 a	281.14 b	11.96 a
LSD 0.05	4.90978108	0.67231906	3.92155631	3.19490171	0.18774143
Red Algae ppm					
0	121.59 c	6.2c	292.75a	257.98c	9.60c
100	151.98 b	8.55 b	275.99b	309.49a	11.88a
300	159.73 a	8.61 a	260.28c	286.8 b	11.31b
LSD 0.05	4.90978108	0.67231906	3.92155631	3.19490171	0.18774143
Interaction between NaCl and Red algae					
0 0	83.29 h	3.07 f	137.61 h	227.99 h	8.16 g
0 100	100.7 g	6.3 e	161.11 g	270.03 e	9.75 e
0 300	123.46 f	7.28 de	200.48 f	289.72 d	9.18 f
50 0	135.56 e	6.4 e	255.6 e	287.62 d	10.42 d
50 100	164.19 c	7.57 d	269.38 d	317.41 c	11.39 c
50 300	174.23 b	10.61 b	301.92 b	326.6 b	13.62 b
100 0	145.91 d	9.12 c	296.02 b	258.35 f	10.23 d
100 100	191.04 a	11.78 a	397.46 a	341.03 a	14.49 a
100 300	181.49 b	7.93 d	278.43 c	244.05 g	11.15 c
LSD 0.05	4.90978108	0.67231906	3.92155631	3.19490171	0.18774143

The same letters in each column indicate no significant differences among different treatments according to the least significant difference LSD test (P≤0.05).

Table 3: Effects of red algae extracts on photosynthetic pigments, malondialhyde content, and electrolyte leakage of common bean plants grown under salt stress.

NaCLmM	Chl. A (mg/g)	Chl. B (mg/g)	Carotenoids (mg/g)	MDA (µM/g F.W)	E.L %
0	1.21 a	0.37 a	0.2 c	8.68 c	26.98 c
50	1.1 b	0.25 b	0.23 b	19.43 b	30.25 b
100	1.11 b	0.26 b	0.26 a	26.62 a	33.44 a
LSD 0.05	0.01899193	0.01742867	0.00763803	0.7178614	1.35262611
Red Algae ppm					
0	1.08 c	0.26 c	0.26 a	26.22 a	35.89 a
100	1.13 b	0.29 b	0.21 b	15.33 b	28.41 b
300	1.21 a	0.34 a	0.21 b	13.19 c	26.36 c
LSD 0.05	0.01899193	0.01742867	0.00763803	0.7178614	1.35262611
Interaction between NaCl and Red algae					
0 0	1.12 c	0.33 c	0.22 c	8.22 g	20.42 g
0 100	1.2 b	0.37 b	0.19 e	9.45 g	27.79 d
0 300	1.32 a	0.42 a	0.2 d	8.38 g	32.72 c
50 0	1.09 c	0.23 e	0.28 a	31.51 b	42.11 b
50 100	1.1 c	0.24 e	0.21 d	14.45 e	26.09 de
50 300	1.12 c	0.28 d	0.19 e	12.34 f	22.55 fg
100 0	1.03 d	0.22 e	0.29 a	38.95 a	45.15 a
100 100	1.09 c	0.25 e	0.25 b	22.09 c	31.36 c
100 300	1.2 b	0.32 c	0.24 bc	18.84 d	23.82 ef
LSD 0.05	0.01899193	0.01742867	0.00763803	0.7178614	1.35262611

The same letters in each column indicate no significant differences among different treatments according to the least significant difference LSD test (P≤0.05).

Table 4: Effects of red algae extracts application on the antioxidant enzymes activities of common bean plants grown under NaCl salt stress.

NaCLmM	POD (sp.activity)	SOD (sp.activity)	APX (sp.activity)	CAT (sp.activity)	PAL (sp.activity)
0	9.33 c	22.71 c	150.07 c	88.24 b	174.83 b
50	19.78 b	43.14 b	297.98 b	243.01 a	353.7 a
100	29.65 a	46.65 a	402.84 a	259.74 a	356.45 a
LSD 0.05	1.36253674	2.53247309	6.40426825	31.277291	24.6466437
Red Algae ppm					
0	10.12 c	22.05 c	174.98 c	84.71 c	234.65 b
100	23.2 b	43.85 b	290.14 b	203.32 b	313.13 a
300	25.45 a	46.59 a	385.77 a	302.96 a	337.2 a
LSD 0.05	1.36253674	2.53247309	6.40426825	31.277291	24.6466437
Interaction between NaCl and Red algae					
0 0	3.3 g	8.56 e	66.33 i	37.87 f	110.89 f
0 100	8.71 f	26.16 d	134.84 h	96.65 de	191.58 e
0 300	15.98 e	33.4 c	249.04 f	130.2 de	222.01 de
50 0	10.25 f	23.19 d	171.77 g	73.51 ef	240.9 d
50 100	22.37 d	47.76 b	305.88 d	152.45 d	364.63 bc

	300	26.73 c	58.48 a	416.29 c	503.07 a	455.58 a
100	0	16.81 e	34.4 c	286.85 e	142.76 d	352.16 bc
	100	38.51 a	57.64 a	429.69 b	360.87 b	383.18 b
	300	33.64 b	47.91 b	491.98 a	275.6 c	334 c
LSD 0.05		1.36253674	2.53247309	6.40426825	31.277291	24.6466437

The same letters in each column indicate no significant differences among different treatments according to the least significant difference LSD test (P≤0.05).

In our study, salt stress treatments significantly decreased plant growth trat is whereas moisture content did was not differed.However, the use of red algae extracts promoted plant growth traits of common bean. In this respect, **[57]**demonstrated that the plant growth and yield of faba bean *(Vicia faba* L.*)* plants treated with *Spirulina Platensis* algae extracts were enhanced compared to the untreated plants or plants grown under different salt stress treatments. The reduction in the plant growth trait sand moisture content can be due to the accumulation of Na^+ and CL^- in plant cells as well as the toxic effects of NaCl under different treatment of saline stress[1].

Phenolic compounds are consideredmajor plant secondary metabolites that have an effective role in plant growth and development as being the chief antioxidant supply present in crops [58].Therefore, the plants containing high contents of antioxidants can show high resistance to the oxidative damage that is caused by salinity stress [59].The current study indicated that the application of red algae extracts significantly increased the total phenolic compounds in plants grown under saline stress. The increase of the total phenolic compounds in plants can be due to the changes in the synthesis of polyphenols that respond to different abiotic factors [60].

In common bean plants, the lower levels of proline under stress have been associated with a better stress resistance of crop cultivars [61]. Proline accumulation is a sensitive physiological index of plant response to various abiotic stresses and plays a positive role in the salt tolerance mechanisms of many crops by contributing to membrane stability **[62]**.Similarly, to the results of our study where we found a significant increase in proline content by using the red algae extracts combined with a high level of salt stress. In this respect, **[63]** reported that the use of algae extracts would increase the proline content of cultivated alfalfa (*Medicagosativa* L.) by 24% compared to the control under salinity stress. Free proline can decrease ROS damage and enhance plant tolerance as well as reduces NaCl saline stress by detoxification of ROS produced because of NaCl toxicity. Further, it may physically extinguish singlet oxygen or respond specifically with hydroxyl radicals [64].

Total soluble sugars contents varied among different genotypes in common bean plants, but it was difficult to assess their role in the salt stress tolerance of the analyzed plants [61].The results of our study showed that the highest total sugar content was recorded at the plants treated with algae extracts under high salt stress. Such increase in the total sugars content might be due to the absorption of the most necessary elements. On the same trend, increases in the carbohydrates with applied of red algae extracts has been reported in our study, it could be due to their important role in the biosynthesis of chlorophyll molecules, which in turn affected total carbohydrates content by increasing photosynthates translocation from source to sink and increasing of different growth substances as well. In this respect, Perveen and Nazir [65]reported an increment in maize carbohydrates and attributed that to the enhancement of photosynthetic efficiency following seaweed application and phytohormones as well as macro and micronutrients. Moreover, the results of this study indicated that there was a significant increase in the free amino acid content with the application of red algae extracts. This increase might be due to the osmotic adjustment where total soluble sugars and free amino acids work as osmolytes to regulate osmotic potential. Meanwhile, Gong, Wen [66]demonstrated that saline stress can affect nitrogen metabolism through affecting the activity of various enzymes.

Seaweed extracts have shown affirmative effects on plants by promoting and improving seed germination, stimulating growth as well as increasing the photosynthetic pigments [67].The results of the present study demonstrated that the usage of red algae extracts increases the content of chlorophyll A and chlorophyll B under the higher salinity levels (50 and100 mM NaCl).Similar results were obtained by **[68]**who showed that U.lactuca extracts increased the total chlorophyll content of cowpea (*Vignaunguiculata*) by 20% when compared to the control treatment. Algae extracts could increase the cellular metabolic rate and delay plant senescence by

protecting and preventing the senescence of chloroplasts, delaying the destruction of chlorophyll and/or increasing the biosynthesis of the latter, because of the high content of N (1.0%) and Fe (0.20%) in the algae extracts, which explains the high rate of photosynthesis in the plant treated with algal extracts **[69]**.

In the present study, lipid peroxidation was estimated as malondialdehyde (MDA) content, thisbeing a biochemical pointer of stress, where it hinders the generation of biomass and decline conceivable outcomes of plant adjustment to push[70]. MDA content in our study at concentrations of 50 and 100 mMNaCl was significantly increased whereas the use ofred algae extracts decreased MDA. The increase of MDA content is a result of lipid peroxidation and membrane deterioration may be attributed to the action of the lipid peroxidase [71, 72]. Further, Ebrahimian and Bybordi [73]attributed this increment of MDA to reactive oxygen species (ROS)that cause membrane lipid peroxidation, selectivity, and reducing membrane fluidity.

The results obtained in this study reported that there were clear differences in electrolyte leakage between red algae extracts and salt stress treatments, except in un-stressed plants where the application of algae extract in both 100 and 300 ppm concentrations significantly increased electrolyte leakage when compared to other stressed plants. Maintaining membrane integrity under saline stress is considered to be an integral part of the salinity tolerance mechanism**[74].** Thus, using red algae extract can maintain the membrane integrity by reducing electrolyte leakage.

Regarding the antioxidant enzymes activities,there is a potent correlation between the tolerance of saline stress and the presence of an effective antioxidant system within the plant and thus resistance to saline stress is closely related to the efficiency of such an antioxidant system **[75].** This is because it is known that plants that grow under saline stress are trying to build a complex and effective antioxidant system as well as ROS scavenging enzymes to beat on the effects of salinity[10]. Moreover, antioxidant enzymes play an important role in maintaining the normal functioning of plants by repairing and detoxifying the oxidative damage caused by stress-generated ROS [2]. The results of the present study are consistent with the findings of Desoky, Ibrahim [1] who found that the activity of the antioxidant enzymes significantly increased in plants under NaCl saline stress. Similarly, Gupta and Pandey [2]indicated that the content of antioxidant metabolites and antioxidant activity enzymes of *Phaseolus vulgaris* L. were increased under saline stress. Therefore, our results recommended that red algae extracts could contribute to detoxifying H_2O_2 by enhancing antioxidant enzymes activity under saline stress. Eventually, the use of red algae extracts mitigated the adverse effects of saline stress, most likely by scavenging ROS and protecting antioxidant enzymes.

5. Conclusions

This study concludes that the exogenous application of red algae extracts enhanced the response of common bean plants (*Phaseolus vulgaris*) grown under salt stress levels (50 and 100 mM NaCl). The beneficial effects of the exogenous use of red algae extracts into common bean plants grown under salinity stress might be ascribed to its protective peroxidation-linked membrane deterioration and scavenging free radicals. In addition, red algae extract can activate the antioxidant system under the normal and saline soil conditions. Moreover, the results of the current study not only allow us to conclude that treating seeds and plants with algae extracts could mitigate the harmful effects of excess NaCl but also could be used as a biological amendment in the soil reclamation technique which can boost food production in the cultivated lands and barren soils accumulated with salt. Eventually, we recommend that the use of red algae extract at 100 ppm is considered the optimal level for plants grown under the high salinity stress (100 mM NaCl) compared to the other levels of the algae extracts.

6. References

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