# Ameliorative Effect of Micro-Algal and Medicinal Plants on Some Biochemical Properties of Bean Plants under Salinity Stress

Emad Abada,<sup>a</sup> Yosra Modafer,<sup>a</sup> Abdullah Mashraqi,<sup>a</sup> Abdel-Rahman M. Shater,<sup>a</sup> Mohamed A. Al Abboud,<sup>a</sup> Mohamed A. Amin,<sup>b</sup> Tarek M. Abdel Ghany,<sup>b,\*</sup> and Hanan A. Said <sup>c</sup>

This study was conducted to examine the ameliorative effects of foliar application of some micro-algal (Chlorella vulgaris and Spirulina platensis) and some medicinal plant leaves (Salix alba, Psidium guajava, and Olea europaea) extracts on Phaseolus vulgaris (Bean) under salinity stress. On a loamy soil, a pots trial was carried out on bean plants grown under salinity stress. Growth characteristics, pigments, osmolytes, total phenol, and antioxidant enzyme contents were determined. S. platensis extract application showed the greatest improvement in shoot length and fresh weight of shoot, which rose 23.5% and 65.1%, respectively compared to the control. The utilized bio-stimulants, particularly S. platensis extracts, remarkably increased the chlorophyll content compared to the control under salinity stress. The photosynthetic pigment, soluble sugars, and soluble protein levels were strengthened by foliar application of biostimulant extract. Proline and antioxidant enzyme levels are significantly reduced using algal and plant extracts treatment. These findings support the treatment's increased contribution to reducing salt stress and their detrimental effects on bean plants. The findings of this study indicate that the use of these biostimulants, especially S. alba, P. guajava, and O. europaea leaf extracts can be considered as an unconventional, ecofriendly, and novel tool in the mitigation of salinity stress.

DOI: 10.15376/biores.18.3.6142-6154

Keywords: Algal; Medicinal plant extracts; Phaseolus vulgaris L.; Biochemical properties; Salinity stress

Contact information: a: Biology Department, College of Science, Jazan University, Jazan 82817, Saudi Arabia; b: Botany and Microbiology Department, Faculty of Science, Al-Azhar University, Nasr City, Cairo 11725, Egypt; c: Botany Department, Faculty of Science, Fayoum University, Fayoum 63514, Egypt; \*Corresponding author: tabdelghany.201@azhar.edu.eg

# INTRODUCTION

Haque *et al.* (2022) stated that in order to assist national economies, it is essential to grow crops and fruits and increase their yields. The great majority of issues that endanger human existence can be solved through agriculture (Abdul Rajak 2022). Crops are directly impacted by soil salinity. As a result of its terrible impact on agricultural areas and decreased crop quality and productivity, salinity is one of the most damaging abiotic stresses (Abdel Latef *et al.* 2020). The accumulation of reactive oxygen species, which results in oxidative stress and causes oxidative damage to proteins, pigments, and DNA in salt-stressed plants, is one of the most serious issues that depend on soil salinity (Shokri-Gharelo and Noparvar 2018). There are numerous disruptions caused by high sodium content in plant cells that reduce plant development (Kumar *et al.* 2020). Reduced

photosynthetic pigments, leaf area, and photosynthetic efficiency were seen in plants exposed to excessive salt concentration (Ashraf and Harris 2013). Moreover, the accumulation of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) from salinity stress leads to oxidative stress, which causes DNA fragmentation, cell shrinkage, and the accumulation of malondialdehyde (MDA), a marker for lipid peroxidation (Zhu et al. 2020). In the authors' study, microalgae was used, which have not garnered enough attention as a potential source of plant biostimulants (Farid et al. 2019). Carillo et al. (2020) stated that algal material extracts or derivatives have long been valued as a resource that can help people and domesticated plants. In recent years, it has been clear that algal formulations can have a variety of impacts on plants, including an increase in biomass, production, and quality. These algal extracts also contain several bioactive chemicals and signaling molecules in addition to minerals and organic elements. Algal extracts are now being used more frequently as plant biostimulants as a non-renewable chemical input in agriculture, as they can help plants flourish in less-than-ideal surroundings like saline situations. The use of plant extracts from Salix alba, Psidium guajava, Olea europaea, and Punica granatum as a medicinal plant to lessen the negative effects of salinity stress on crop growth and biochemical constituents of common bean plants has not been reported, but many extracts from the other plants have been used to enhance the growth of other crops. Agbagwa (2014) investigated how well Senna alata crude extracts improved Celosia argentea vegetative and reproductive growth. He demonstrated how soaking seeds in the extract prior to planting boosted and improved seedling length, leaf area, dry weight, and leaf area ratio. The current study intends to investigate how extracts from medicinal herbs and algae (Chlorella vulgaris, Spirulina platensis, S. alba, P. guajava, and O. europaea) can reduce the stress caused by salinity on common bean plants. This study investigates how extracts from medicinal plants and algae alter some metabolic constituents of common bean plants. Additionally, it makes evident the importance of a novel class of biostimulants (S. alba, P. guajava, and O. europaea leave extracts), which have a protective effect against salinity stress. However, it also highlights the pressing need to address the underlying processes underlying these benefits.

### EXPERIMENTAL

### **Field Experiment**

During the spring of 2020 to 2021, a pot experiment was conducted with loamy soil. Prior to planting, a surface soil sample (0 to 30 cm) from the experimental area was taken for investigation, and some physical and chemical parameters were determined using the reported methods (Klute 2018). The data of analyses are presented in Table 1.

The experimental treatments were arranged within the sample groups in a completely randomized block design. The studied area was divided into 6 groups; the area of each group was tested in 6 replicates. *Phaseolus vulgaris* L. var. Bronco seeds were sown in hills (2 seeds bill<sup>-1</sup>) at soil depth of 2 to 3 cm. Additionally, ammonium nitrate (33.5% N) was added at the rate of 50 kg N fed.<sup>-1</sup>, after 23 days of planting. All plots were irrigated at 200 ppm of sodium chloride as a control. Algae and medicinal plant tissues were extracted in sterile distilled water at a ratio of 1:200 (w/v) for 45 min at 60 °C. The extracts were kept at 4 °C for field testing purposes and filtered through a filter paper (Anisimov *et al.* 2013). At 20 and 35 days of sowing, the extracts were sprayed on the leaves as a foliar treatment at a dosage of 5 g of powdered medicinal plants or algae/L (400 L·fed<sup>-1</sup>). A preliminary

experiment in which they induced the highest percentage of germination led to the selection of the extracts' concentration. The experiment included six treatments as follows: (1) Control (saline soil without any foliar of algal and medicinal plant), (2) Algal extract of *C. vulgaris*, (3) Algal extract of *S. platensis*, (4) Medicinal plant extract of *S. alba*, (5) Medicinal plant extract of *P. guajava*, and (6) Medicinal plant extract of *O. europaea*. Analysis of material extracts are shown in Table 2 as mentioned previously in other studies.

	Particle-Size Distribution (%)					pH (1: 2.5 in suspension)					7.20				
	C. Sand					2	0.0	EC (dS/m, in soil paste)						3.38	
	F. Sand				1	8.1	Soluble lons (meq/L) Ca				Ca++	4.8			
	Silt				4	0.6	Mg				Mg <sup>++</sup>	2.3			
	Clay					2	1.3							Na⁺	96.4
	Texture class					Lo	amy							K+	0.3
	O.M (%)					0	.44							CO32-	-
	CaCO <sub>3</sub> (%)					1	.42	HCO <sub>3</sub> -				1.7			
	SP (%)					3	5.0	Cl				92.7			
	W.H.C (%)						33	SO4 <sup>2-</sup>				9.4			
	Available nutrient (mg/kg)														
Ν	23.8	Ρ	9.34	Mn	0.39	Fe	0.43	Zn	0.13	Fe	0.43	Cu	1.37	7 K	123.0

### Table 2. Analysis of Material Extracts

Extracts Species	Analysis	Reference
C. vulgaris	According to a chromatographic analysis, the main phenolic constituents were gallic, caffeic, <i>p</i> -coumaric, and ferulic corrosives.	(Wan <i>et</i> <i>al.</i> 2019)
S. platensis	Analysis by gas chromatograph-/mass spectrometry (GC-MS) found that 9,12-Hexadecanoic acid, 15-octadecadienoic acid methyl ester, and 9,12- octadecatrienoic acid methyl ester are all present in methanol extract in amounts of 29%, 24%, and 24.36%, respectively.	(Deyab <i>et</i> <i>al.</i> 2021)
S. alba	Analysis by ultra-performance liquid chromatography photodiode detector-quadrupole/time-of-flight mass spectrometry (UPLC-PDA-Q/TOF-MS) showed that 29 phenolic compounds were found in the leaves and, there were 5575.96 mg of phenolic compounds overall per 100 g of dry weight (DW).	(Piątczak <i>et al.</i> 2020)
P. guajava	GC-MS analysis of the essential oil showed that the dominant chemical compounds present in the leaf oil were iso- caryophyllene (33.53%), veridiflorene (13%), farnesene (11.65%), dl-limonene (9.84%), d-cadinene (1.75%), a-copaene (2.80%), a-humulene (3.74%), aromadendrene (1.70%), and scadinol (0.08%).	(Weli <i>et al.</i> 2019)
O. europaea	High-Performance Liquid Chromatography Coupled with Diode-array Detection and Electrospray Ionization Tandem Mass Spectrometry (HPLC/DAD/MS) analysis indicated that hydroxytyrosol and flavonoids, including rutin, luteolin-7- glucoside, verbascoside, and oleuropein were the major phenolic components in leaves.	(Silva et al. 2006)

*Phaseolus vulgaris* L. var. Bronco-treated plant samples (six plants) were randomly selected after 45 days of seeding to examine their morphological plant traits (lengths of shoot, fresh, and dry weights of shoot, and number of leaves). Fresh leaf pigments, dry shoot carbohydrates, protein, phenol, and proline, as well as the antioxidant enzyme content of apical buds, were all subject to biochemical investigation. The number of biochemical determinations that were made, and the nature of those determinations are described below.

### Estimation of pigments and carotenoids

Vernon and Seely (2014) developed a technique for measuring the pigments in plants. Approximately 1 g of leaves was chopped into tiny pieces. The fragments were blended for 2 min in 100 mL of 80% acetone. The mixture was transferred quantitatively and filtered using Whatman No. 1 filter paper using a Buchner filter. The filtrate was poured into a 100-mL volumetric flask, and 100 mL of acetone at 80% was then added to the flask. Estimated chlorophylls "a" and "b" (Lichtentahler 1989).

#### Estimation of total soluble carbohydrates and protein

One gram of the dried plant tissues was extracted in 5 mL of phenol solution (2%) and 10 mL of trichloroacetic acid solution (30%) to determine the amount of carbohydrates present. 2 mL of the extract was added to 4 mL of anthrone reagent, the green-blue color was determined at 620 nm Umbreit *et al.* (1957)

The dried shoot's soluble protein content was evaluated by Lowry *et al.* (1951). The dried material (0.1 g) was homogenized with 5 mL of phenol solution (2%) and 10 mL of distilled water. The acquired extract was combined with 5 mL of alkaline reagent {containing: 50 mL from solution A (50 mL of 2% sodium carbonate and dissolved in 0.1 N sodium hydroxide): 1 mL from solution B (0.5 g copper sulfate dissolved in 1.0% potassium sodium tartrate)} and homogenized thoroughly. Then, 0.5 mL of the diluted folin phenol reagent (1:3 v/v) was added. After 30 min, the developed color at 750 nm was measured.

### Estimation of total phenols

The colorimetric method of Folin-Denis as described by Daniel and George (1972) was used. One gram of dry plant were extracted with 5 to 10 mL 80% ethanol for at least 24 h at 0 °C. The alcohol was clarified, the remaining residue was re-extracted with 5 to 10 mL 80% ethanol 3 times. At the end, the clarified extract was completed to 50 mL using 80% ethanol. The total phenolic constituents were estimated using the Folin-Ciocalteu method. An aliquot of 0.5 mL of the previous extract and 0.5 mL of Folin-Denis reagent were well mixed in a dry test tube, the tube was thoroughly shaken for 3 min. 1.0 mL of saturated Na<sub>2</sub>CO<sub>3</sub> solution was added, mixed well, and 3 mL of distilled water were added. After one hour, the development colour was read at 725 nm.

### Calculation of free proline

According to Bates *et al.* (1973), the proline content in the dried shoot samples was evaluated. Briefly, 10 mL of sulfosalicylic acid (3%), together with a half gram of the dried material, were combined. The mixture was filtered, and 2 mL of the filtrate was then added to 2 mL of acid ninhydrin, which was made by heating 1.25 ml of ninhydrin with 30 mL of glacial acetic acid and 20 mL of 6 M phosphoric acid for one hour. The reaction was continued in an ice bath, and the preceding combination was then given 4 mL of toluene.

The absorbance of the produced color was measured at 520 nm. On the basis of a developed standard curve with known L-proline concentration, proline contents in the sample were identified.

### Extraction of enzymes catalase, peroxidase, and polyphenol oxidase

Young leaves and terminal buds have antioxidant enzymes such as peroxidase, catalase, superoxide dismutase (SOD) and polyphenol oxidase. According to this procedure, two grams of plant buds were mixed with 10 mL of phosphate buffer (0.1 M, pH 6.8), and the mixture was centrifuged while chilling for 20 min at 20,000 rpm. The enzyme source was the clear supernatant, which contained the enzymes (Mukherjee and Choudhuri 1983) and assessed as follows.

Catalase (CAT) activities were calculated as mentioned by Aebi (1984). For catalase (CAT, EC 1.11.1.6) activity, 10 mL of the whole mixture (40 L of the enzyme extract + 9.96 mL of pH 7.0 oxygen water phosphate buffer; 0.16 mL  $H_2O_2$  (30%) + 100 mL 50 mM phosphate buffer) was used. Using UV-colorimetry at 250 nm, the change in H2O2 absorbance after 60 seconds was used to determine the catalase enzyme activity.

Peroxidase (POX) activities were estimated according to Castillo *et al.* (1984). The test solution (5 mL) for peroxidase activity (POD, EC 1.11.1.7) contained the following ingredients:  $50 \,\mu\text{M}$  pyrogallol +  $50 \,\mu\text{M}$  H<sub>2</sub>O<sub>2</sub> + 125  $\mu\text{M}$  of phosphate buffer (pH 6.8) + 1.0 mL of the 20 × diluted enzyme extract. At an absorbance of 420 nm, the color created as a result of the purpurogallin concentration was determined. The peroxidase enzyme's activity was measured in enzyme units (EU)/mg protein.

Polyphenoloxidase (PPO) activity was evaluated according to the method of Matta and Diamoned (1963). In a nutshell, the solution assay entails the mixing of 100  $\mu$ mol of pyrogallols with 125  $\mu$ mol of phosphate buffer (pH 6.8) and 2 mL of crude enzyme extract, followed by 5 min of incubation at 25 °C. The reaction was halted after the incubation period by adding 1.0 mL of 5% H<sub>2</sub>SO<sub>4</sub> to the mixture. The above-mentioned procedures were used on the blank control sample. At 430 nm, the developed color was measured in the treated and control solutions. The difference between two optical densities/g of fresh weight/hour was used to calculate the enzyme activity.

The activity of superoxide dismutase (SOD) was estimated according to methods described by Marklund and Marklund (1974). In this method, 10 mL test solution containing the following ingredients was used: 3.6 mL of pure water, 5.5 mL of 50 mM phosphate buffer (pH 7.8), 0.1 mL of enzyme extract, and 0.8 mL of 3 mM pyrogallol (dissolved in 10 mM HCl). Pyrogallol's decrease percentage was determined at 325 nm. An EU mg-1 protein was required to prevent 50% of the autoxidation of pyrogallol at 25 °C in order to evaluate the activity of SOD.

### **Statistical Analysis**

At the 0.05 level of probability, statistical calculations were carried out using the computer programs Microsoft Excel version 365 (Microsoft Corp., Redmond, WA, USA) and SPSS v.25 (statistical package for the social science version 25.0) (Snedecor and Cochran 1980) (SPSS Inc., Chicago, IL, USA). To analyze the variance of quantitative data with a parametric distribution, one-way analysis of variance (ANOVA) and *post hoc* Tukey test were utilized. The confidence interval was set at 95%, while the allowed margin of error was set at 5%.

# **RESULTS AND DISCUSSION**

# Algal and Medicinal Plants Extract Improved Morphological Parameters of Common Bean under Saline Soil Stress

Through pleiotropic pathways involving osmotic stress, nutritional imbalance, ion toxicity, and oxidative stress, salinity adversely impacts plant growth, development, and yield (Ferchichi *et al.* 2018). It has been demonstrated that salinity (salt stress) negatively affects common bean plants by lowering shoot height, fresh weight, and leaf count. Salinity may have a growth-reducing effect on common bean plants for a variety of reasons, including severe osmotic stress and ion toxicity (Chung *et al.* 2020). Salinity stress can also prevent cell division, cell elongation, and cell expansion, as indicated by Radi (2013). The use of natural plant extracts to support the growth of various crop plants, such as maize under salt stress, has been documented (Abdel Latef *et al.* 2019).

Data in Table 3 show that foliar application of algal (*S. platensis*) and medicinal plant (*O. europaea*) extracts caused significant increase of growth parameters of common bean under study compared to the control and other treatments. *S. platensis* extract appeared to cause the highest improvement of shoot length and fresh weight of shoot by 23.5% and 65.1%, respectively, rather than the control. *S. platensis* extract's stimulating effects were evident as a result of its abundance in contents of proteins, minerals, trace elements, and numerous vitamins, including B1, B2, B12, and E (Sall *et al.* 1996).

The common bean culture trial's findings revealed that plants treated with *S. platensis* algae extract had generally improved the growth parameters. In this concern, Farooq *et al.* (2017) observed that the efficiency of wheat under salt stress was improved by the foliage application of all plant extracts (sorghum extract, common bean extract, brassica extract, and moringa extract), as evidenced by improvements in grain weight, grain number, and grain yield.

Treatments	Shoot Length (cm)	Fresh Weight of Shoot (g pol <sup>-1</sup> )	Dry Weight of Shoot (g plant <sup>-1</sup> )	Number of Leaves (g plant <sup>-1</sup> )	
Control	22.76 ± 0.21b	1.72 ± 0.03c	0.143 ± 0.01b	4.34 ± 0.002b	
C. vulgaris	24.95 ± 0.36ab	2.10 ± 0.04bc	0.171 ± 0.02a	4.66 ± 0.004a	
S. platensis	28.11 ± 0.25a	2.93 ± 0.02a	0.182 ± 0.02a	4.83 ± 0.003a	
S. alba	23.48 ± 0.45b	2.44 ± 0.06b	0.150 ± 0.04b	4.66 ± 0.010a	
P. guajava	22.15 ± 0.41b	2.62 ± 0.12b	0.165 ± 0.02a	4.50 ± 0.020b	
O. europaea	26.03 ± 0.22a	2.84 ± 0.02a	0.173 ± 0.03a	4.70 ± 0.005a	
LSD <sub>0.05</sub>	3.214	0.952	0.21	0.291	

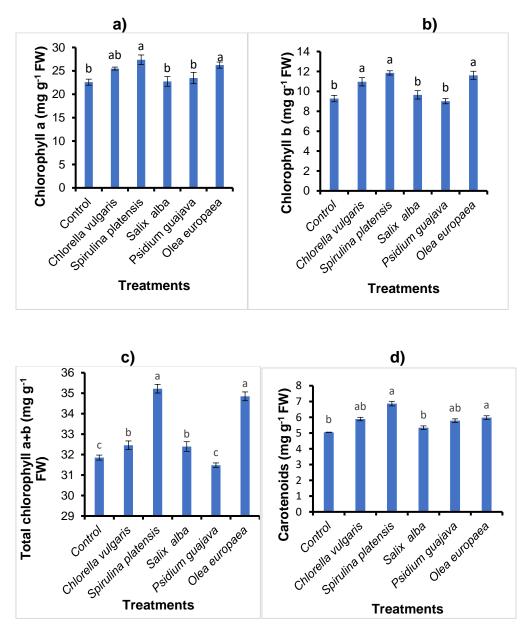
**Table 3.** Effects of Algal and Medicinal Plants Extraction on Shoot Lengths, Freshand Dry Weights of Shoot, and Number of Leaves of *Phaseolus vulgaris* L. var.Bronco under Saline Soil Stress

Data represents mean  $\pm$  standard error (n = 10). Different lowercase letters in the same species within a column indicate significant differences ( $P \le 0.05$ ) by Tukey's test (LSD).

# Biochemical Parameters of *Phaseolus vulgaris* L. var. Bronco Affected by Algal and Medical Plant Extracts

### Pigments contents

One of the major determinants of photosynthetic capacity is the amount of chlorophyll. The data in this study (Fig. 1) show that, in comparison to those found with other extras applied, salt stress caused a considerable decrease in the amounts of carotenoid and chlorophyll content in common bean leaves.



**Fig. 1.** Effects of algal and medicinal plant extracts on chlorophyll a, chlorophyll b, chlorophyll (a + b), and carotenoids (d) content of *P. vulgaris* L. plants under saline soil stress. (Bars represent means and standard errors of three independent replications (n = 3). Different letters indicate significant differences among the treatments at *P* < 0.05, according to LSD test.

Results in Fig. 1 confirmed that under salinity conditions, the use of biostimulants, especially *Spirulina platensis* and *O. europaea* extracts, significantly improved the chlorophyll and carotenoid contents compared with the stress sample of bean plant. This may be because seaweed and plant extracts include active ingredients including betaine, amino acids, and other compounds that prevent the degradation of chlorophyll (Blunden *et al.* (1996).

Other results, such as those in Fig. 1, also demonstrate that the use of biostimulants can mitigate the harm that salinity causes to the chlorophyll concentrations of common bean plants. The decrease in leaf area, which is crucial for catching light and enabling photosynthesis, may be the cause of the decline in photosynthetic pigments. Moreover, under abiotic stress circumstances, increases in the activity of chlorophyll-degrading enzymes including chlorophyllase caused declines in photosynthetic pigments (Abdel Latef *et al.* 2020). The high percentage of protein, Ca, Mg, Fe, and Zn ions, which have a direct impact on photosynthetic pigments because Ca and Fe decrease in chlorophyll biosynthesis, and vitamin E, which acts as an antioxidant, are all present in *S. platensis* extract, which may account for its protective effect on the extent of photosynthetic pigment and carotenoids. It was also shown to be connected to a variety of biological processes in the plant as an electron transport source and receptor and an enzyme catalyst (Abdel Latef *et al.* 2019).

### **Metabolite Contents**

Data in Table 4 show that salinity stress significantly decreased the content of soluble carbohydrates, protein contents of shoot of common bean, and applications of biostimulants significantly enhanced the soluble carbohydrates, protein contents. The highest value was obtained with medicinal plant extract of *Psidium guajava* treatment, which caused relative increase of soluble sugars and protein content by 173% and 36.1%, respectively, over the control (Table 4).

Proline has the highest water solubility and exists in a zwitter ionic state. Proline shares this property with other compounds. It is collectively referred to as "compatible solutes" that are accumulated in the wide range of organisms to adjust cellular osmolality. The results from this study showed a significant increase in proline and phenol contents in response to salinity stress.

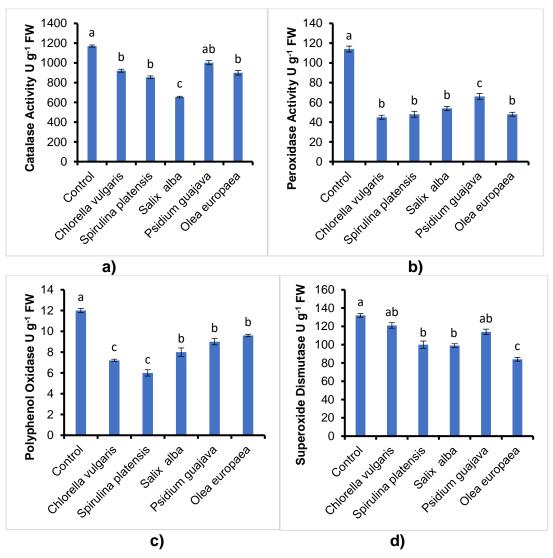
The accumulation of phenolic compound under abiotic stresses helps in the stabilization of the subcellular/non-photosynthetic membranes by detoxifying the reactive oxygen species, thus improving the osmotic adjustment (Farooq *et al.* 2009). The salinity stress in the presence of algal and medicinal plant extracts show a significant decrease in proline content. These findings support the stronger contribution from this treatment in reducing the detrimental effects of salt stress on common plants. Phenolics in algal and medicinal plant extracts may be responsible for the improved *P. vulgaris* performance (Jabran and Farooq 2013). Eventually, the common bean performance under stress circumstances was improved by the plant and algal extracts as a result of these extract treatments that increase water and nutrient uptake, enzyme activity, photosynthesis, gene expression, and signal transmission by modulating the metabolism of phytohormones (Macías *et al.* 2007).

**Table 4.** Effects of Algal and Medicinal Plants Extract Soluble Carbohydrates,

 Protein, Phenol, and Proline Contents of *P. vulgaris* L. Plants in Saline Soil

Treatments	Soluble Carbohydrates	Soluble Protein	Total Phenol	Total Proline	
		(mg/g Dry W	ight)		
Control	8.73 ± 0.21c	16.79 ± 0.54c	0.353 ± 0.07b	2.94 ± 0.15a	
C. vulgaris	14.68 ± 0.36b	21.39 ± 0.31a	0.106 ± 0.06c	2.17 ± 0.09b	
S. platensis	16.50 ± 1.3ab	19.88 ± 0.42ab	0.294 ± 0.11b	1.18 ± 0.25d	
S. alba	19.85 ± 0.65ab	20.97 ± 0.75a	0.169 ± 0.03c	2.14 ± 0.21b	
P. guajava	23.88 ± 0.74a	22.22 ± 0.65a	0.473 ± 0.04a	1.94 ± 0.32c	
O. europaea	13.51 ± 1.32b	18.70 ± 0.63b	0.546 ± 0.05a	1.86 ± 0.24c	
LSD 0.05	4.21	1.45	0.125	0.354	

Data represent means  $\pm$  standard error (n = 3). Different lowercase letters in the same species within a column indicate significant differences ( $P \le 0.05$ ) by Tukey's test (LSD).



**Fig. 2.** Effects of algal and medicinal plant extracts on Catalase, Peroxidase, Polyphenol oxidase, and Superoxide dismutase content of common bean plants. (Bars represent means and standard errors of three independent replications (n = 3). Different alphabetical letters indicate significant differences among the treatments at P < 0.05, according to LSD test.

### **Enzyme Activities**

The findings in Fig. 2 demonstrate the effects of salinity stress on the antioxidant enzyme activities that take part in scavenging reactive oxygen species (catalase, peroxidase, superoxide dismutase, and polyphenol oxidase) (ROS).

In comparison to foliar application of algal and medicinal plant extracts, the results revealed a considerable increase in the activities of catalase, peroxidase, superoxide dismutase, and polyphenol oxidase in the leaves of the apical tip of common bean plants under salinity treatment (control). Because of the increased accumulation of ROS, particularly O<sub>2</sub>, in chloroplasts, mitochondria, and peroxisomes, salinity stress is eventually linked to enhanced oxidative stress. Plants typically use the stimulation of antioxidant enzyme activity as a defense mechanism against oxidative stress (Foyer and Noctor 2003). Additionally, the current findings in Fig. 2 demonstrate that a biostimulant might lessen the harm that oxidative stress produces to common bean plants by salt stress. Antioxidant enzymes may be drastically decreased by the addition of biostimulants; this might be because of the several osmo-protectant molecules present in biostimulants, which lessen the negative consequences of salinity stress.

# CONCLUSIONS

- 1. The results of this study showed that natural agents play a significant impact in reducing the negative effects of salt stress on *P. vulgaris* L.
- 2. *Spirulina platensis* extract showed the greatest improvement in growth parameters and pigment levels compared to the stress sample.
- 3. Bio-stimulants, particularly *S. platensis* extracts, dramatically increased the levels of protein, phenol, total soluble carbohydrates, and chlorophyll compared to the control.
- 4. Algal and medicinal plant extracts showed significant decrease in proline and antioxidant enzymes' content. These findings support the treatment's larger contribution in reducing detrimental effects of saltwater stress on *P. vulgaris* L.
- 5. These results confirm the promising role of biofertilizers in stimulating growth and protecting against salt stress as an alternative way to chemical treatment.
- 6. This research emphasizes the bioprotective benefits against saline stress that agricultural biofertilizers may provide to make agriculture more sustainable, environmentally friendly, and robust as well as a replacement for synthetic protectants, which are eroding in consumer favor. Additionally, it makes evident the importance of a novel class of biostimulants (extracted from *S. alba, P. guajava*, and *O. europaea* leaves), which have a protective effect against abiotic stress. However, it also highlights the pressing need to address the underlying processes underlying these benefits.

### **Conflicts of Interest**

The authors declare no conflict of interest

# Funding

This research was funded by Deputyship for Research Innovation, Ministry of Education in Saudi Arabia, through project number ISP22-18.

### ACKNOWLEDGMENTS

The authors extend their appreciation to the Deputyship for Research Innovation, Ministry of Education in Saudi Arabia, for funding this research work through project number ISP22-18.

# **REFERENCES CITED**

- Abdel Latef, A. A. H., Abu Alhmad, M. F., Kordrostami, M., Abo–Baker, A.-B. A.-E., and Zakir, A. (2020). "Inoculation with *Azospirillum lipoferum* or *Azotobacter chroococcum* reinforces maize growth by improving physiological activities under saline conditions," *Journal of Plant Growth Regulation* 39(3), 1293-1306. DOI: 10.1007/s00344-020-10065-9
- Abdel Latef, A. A. H., Mostofa, M. G., Rahman, M. M., Abdel-Farid, I. B., and Tran, L.-S. P. (2019). "Extracts from yeast and carrot roots enhance maize performance under seawater-induced salt stress by altering physio-biochemical characteristics of stressed plants," *Journal of Plant Growth Regulation* 38(3), 966-979. DOI: 10.1007/s00344-018-9906-8
- Abdul Rajak, A. R. (2022). "Emerging technological methods for effective farming by cloud computing and IoT," *Emerging Science Journal* 6(5). DOI:10.28991/ESJ-2022-06-05-07
- Aebi, H. (1984). "Catalase *in vitro*," in: *Methods in Enzymology*, L. Packer (Ed.), Academic Press, San Diego, CA, USA, pp. 105, 121-126. DOI: 10.1016/S0076-6879(84)05016-3
- Agbagwa, I. O. (2014). "Crude extracts of Senna alata (L.) Roxb. mimics plant growth hormones in promotion of vegetative and reproductive growth in Celosia argentea L.," American Journal of Plant Sciences 05(13), 1918-1925. DOI: 10.4236/ajps.2014.513205
- Anisimov, M. M., Skriptsova, A. V., Chaikina, E. L., and Klykov, A. G. (2013). "Effect of water extracts of seaweeds on the growth of seedling roots of buckwheat (*Fagopyrum esculentum* Moench)," *International Journal of Research and Reviews in Applied Sciences* 16(2), 282-287.
- Ashraf, M., and Harris, P. J. C. (2013). "Photosynthesis under stressful environments: An overview," *Photosynthetica* 51(2), 163-190. DOI: 10.1007/s11099-013-0021-6
- Bates, L. S., Waldren, R. P., and Teare, I. D. (1973). "Rapid determination of free proline for water-stress studies," *Plant and Soil* 39(1), 205-207. DOI: 10.1007/BF00018060
- Blunden, G., Jenkins, T., and Liu, Y.-W. (1996). "Enhanced leaf chlorophyll levels in plants treated with seaweed extract," *Journal of Applied Phycology* 8(6), 535-543. DOI: 10.1007/BF02186333
- Carillo, P., Ciarmiello, L. F., Woodrow, P., Corrado, G., Chiaiese, P., and Rouphael, Y. (2020). "Enhancing sustainability by improving plant salt tolerance through macroand micro-algal biostimulants," *Biology* 9(9), article 253. DOI: 10.3390/biology9090253
- Castillo, F. J., Penel, C., and Greppin, H. (1984). "Peroxidase release induced by ozone in sedum album leaves," *Plant Physiology* 74(4), 846-851. DOI: 10.1104/pp.74.4.846
- Chung, Y. S., Kim, K.-S., Hamayun, M., and Kim, Y. (2020). "Silicon confers soybean resistance to salinity stress through regulation of reactive oxygen and reactive nitrogen species," *Frontiers in Plant Science* 10, article 1725. DOI:

10.3389/fpls.2019.01725

- Daniel, H. D., and George, C. (1972). "Peach seed dormancy in relation to endogenous inhibitors and applied growth substances," *Journal of the American Society for Horticultural Science* 97(5), 651-654. DOI: 10.21273/JASHS.97.5.651
- Deyab, M. A., El-Sheekh, M. M., Hasan, R. S. A., Elsadany, A. Y., and Abu Ahmed, S. E. S. (2021). "Phytochemical components of two cyanobacterial local strains," *Scientific Journal for Damietta Faculty of Science* 11(1), 67-75. DOI: 10.21608/sjdfs.2021.195593
- Farid, R., Mutale-Joan, C., Redouane, B., Mernissi Najib, E., Abderahime, A., Laila, S., and Arroussi Hicham, E. (2019). "Effect of microalgae polysaccharides on biochemical and metabolomics pathways related to plant defense in *Solanum lycopersicum*," *Applied Biochemistry and Biotechnology* 188(1), 225-240. DOI: 10.1007/s12010-018-2916-y
- Farooq, M., Rizwan, M., Nawaz, A., Rehman, A., and Ahmad, R. (2017). "Application of natural plant extracts improves the tolerance against combined terminal heat and drought stresses in bread wheat," *Journal of Agronomy and Crop Science* 203(6), 528-538. DOI: 10.1111/jac.12214
- Farooq, M., Wahid, A., Kobayashi, N., Fujita, D., and Basra, S. M. A. (2009). "Plant drought stress: Effects, mechanisms and management," *Agronomy for Sustainable Development* 29(1), 185-212. DOI: 10.1051/agro:2008021
- Ferchichi, S., Hessini, K., Dell'Aversana, E., D'Amelia, L., Woodrow, P., Ciarmiello, L. F., Fuggi, A., and Carillo, P. (2018). "*Hordeum vulgare* and *Hordeum maritimum* respond to extended salinity stress displaying different temporal accumulation pattern of metabolites," *Functional Plant Biology* 45(11), 1096-1109. DOI: 10.1071/FP18046
- Foyer, C. H., and Noctor, G. (2003). "Redox sensing and signalling associated with reactive oxygen in chloroplasts, peroxisomes and mitochondria," *Physiologia Plantarum* 119(3), 355-364. DOI: 10.1034/j.1399-3054.2003.00223.x
- Haque, I., Alim, M., Alam, M., Nawshin, S., Rashed, Sh., Noori, H., and Habib, T. (2022). "Analysis of recognition performance of plant leaf diseases based on machine vision techniques," *Journal of Human, Earth, and Future* 3(1), 129. DOI: 10.28991/HEF-2022-03-01-09
- Jabran, K., and Farooq, M. (2013). "Implications of potential allelopathic crops in Agricultural systems," in: *Allelopathy*, Springer Berlin Heidelberg, Germany, pp. 349-385. DOI: 10.1007/978-3-642-30595-5\_15
- Klute, A. (2018). "Water retention: Laboratory methods," In: *Methods of Soil Analysis: Part 1 Physical and Mineralogical Methods*, Vol. 5, Wiley Online Library, pp. 635-662. DOI: 10.2136/sssabookser5.1.2ed.c26
- Kumar, A., Singh, S., Gaurav, A. K., Srivastava, S., and Verma, J. P. (2020). "Plant growth-promoting bacteria: biological tools for the mitigation of salinity stress in plants," *Frontiers in Microbiology* 11, article 1216. DOI: 10.3389/fmicb.2020.01216
- Lichtentahler (1989). The Science of Photobiology, K. C. Smith (ed.), Springer, Stanford University School of Medicine, Stanford, CA, USA. DOI: 10.1007/978-1-4615-8061-4
- Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951). "Protein measurement with the Folin phenol reagent," *Journal of Biological Chemistry* 193(1), 265-275. DOI: 10.1016/S0021-9258(19)52451-6
- Macías, F. A., Molinillo, J. M., Varela, R. M., and Galindo, J. C. (2007). "Allelopathy— A natural alternative for weed control," *Pest Management Science* 63(4), 327-348.

DOI: 10.1002/ps.1342

- Marklund, S., and Marklund, G. (1974). "Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase," *European Journal of Biochemistry* 47(3), 469-474. DOI: 10.1111/j.1432-1033.1974.tb03714.x
- Matta, A., and Diamoned, C. (1963). "Symptoms of Fusarium wilt in relation to quantity of fungus and enzyme activity in tomato stems," *Phytopathology* 53(5), 574–587.
- Mukherjee, S. P., and Choudhuri, M. A. (1983). "Implications of water stress-induced changes in the levels of endogenous ascorbic acid and hydrogen peroxide in Vigna seedlings," *Physiologia Plantarum* 58(2), 166–170. DOI: 10.1111/j.1399-3054.1983.tb04162.x
- Piątczak, E., Dybowska, M., Płuciennik, E., Kośla, K., Kolniak-Ostek, J., and Kalinowska-Lis, U. (2020). "Identification and accumulation of phenolic compounds in the leaves and bark of *Salix alba* (L.) and their biological potential," *Biomolecules* 10(10), article 1391. DOI:10.3390/biom10101391
- Radi, A. (2013). "Physiological and biochemical responses of salt-tolerant and saltsensitive wheat and bean cultivars to salinity," *J. Biol. Earth Sci.* 3(1), B72-B88.
- Sall, M. G., Dankako, B., Badiane, M., Ehua, E., and Kuakuwi, N. (1996). "Results of a nutritional rehabilitation trial with *Spirulina* in Dakar (about 59 cases)," *Black African Medicine* 46, 143-146.
- Shokri-Gharelo, R., and Noparvar, P. M. (2018). "Molecular response of canola to salt stress: Insights on tolerance mechanisms," *PeerJ* 6, article ID e4822. DOI: 10.7717/peerj.4822
- Silva, S., Gomes, L., Leitao, F., Coelho, A. V, and Boas, L. V. (2006). "Phenolic compounds and antioxidant activity of *Olea europaea* L. fruits and leaves," *Food Science and Technology International* 12(5), 385-395. DOI: 10.1177/1082013206070166
- Snedecor, G. W., and Cochran, W. G. (1980). *Statistical Methods*, 7<sup>th</sup> Ed., Iowa State University Press, Ames, IA, USA.
- Umbreit, W. W., Burris, R. H., and Stauffer, J. F. (1957). "Manometric techniques. A manual describing methods applicable to the study of tissue metabolism," in: *Manometric Techniques. A Manual Describing Methods Applicable to the Study of Tissue Metabolism*, Burgess Publishing Co., Minneapolis, MN, USA.
- Vernon, L. P., and Seely, G. R. (2014). *The Chlorophylls*, Academic Press, Cambridge, MA, USA.
- Wan, M. A. W. M., Lorwirachsutee, A., Theodoropoulos, C., and Gonzalez- Miquel, M. (2019). "Polyol-based deep eutectic solvents for extraction of natural polyphenolic antioxidants from *Chlorella vulgaris*," ACS Sustain. Chem. Eng. 7(5), 5018-5026. DOI:10.1021/acssuschemeng.8b05642
- Zhu, J., Fan, Y., Shabala, S., Li, C., Lv, C., Guo, B., Xu, R., and Zhou, M. (2020).
  "Understanding mechanisms of salinity tolerance in barley by proteomic and biochemical analysis of near-isogenic lines," *International Journal of Molecular Sciences* 21(4), article 1516. DOI:10.3390/ijms21041516

Article submitted: April 1, 2023; Peer review completed: July 8, 2023; Revisions received and accepted: July 20, 2023. Published: July 25, 2023. DOI: 10.15376/biores.18.3.6142-6154