Egypt. J. Bot. **Vol. 63**, No. 2, pp. 607-620 (2023)

Egyptian Journal of Botany
http://ejbo.journals.ekb.eg/

The Influence of *Spirulina platensis* on Physiological Characterization and Mitigation of DNA Damage in Salt-stressed *Phaseolus vulgaris* L. Plants

Mohamed A. Taha[(1)], H.R. Moussa[(2)#], Eldessoky S. Dessoky[(3, 4)]
[(1)]Department of Horticulture, Faculty of Agriculture, Menoufia University, Menoufia, Egypt; [(2)]Radioisotope Department, Nuclear Research Center, Atomic Energy Authority, Dokki, Giza 12311, Egypt; [(3)]Department of Plant Genetic Transformation, Agricultural Genetic Engineering Research Institute, Agricultural Research Center, P.O. Box, 12619, Giza, Egypt; [(4)]Department of Biology, College of Science, Taif University, P.O. Box 11099, Taif 21944, Saudi Arabia.

SALINITY severely reduced crop productivity. In this work, the effectiveness of *Spirulina platensis* (100mg/L) as a foliar growth stimulator was tested for its ability to reduce the harmful effect of salinity on *Phaseolus vulgaris* L. plants. The experiment was divided into four groups as follows: G_1 (control), G_2 (200mM NaCl), G_3 (100mg/L of *Spirulina platensis*), and G_4 (200mM NaCl + 100mg/L of *Spirulina platensis*). The results declared that shoot weight, plant height, leaves number, during the vegetative growth stage, pods number/plant, seed number/pod, pods weight during fruiting stage, as well as content of carotenoids, chlorophyll *a*+*b*, 100-seed weights, photosynthetic activity (assimilation of $^{14}CO_2$), and total protein content reduced considerably in salt-stressed *Phaseolus vulgaris*. When compared to salt-stressed plants, the above metrics were enhanced by G_4. In comparison to the control, the presence of salinity increased the activities of catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPX), peroxidase activity (POD), malondialdehyde (MDA), free proline, transpiration rate, and total phenol. Additionally, compared to plants under salt stress, G_4 application (200mM NaCl + 100mg/L of *Spirulina platensis*) decreased the aforementioned metrics. Treatments of *Phaseolus vulgaris* with 200mM NaCl + 100mg/L of *Spirulina platensis* considerably increased the macronutrient content and decreased the Na^+ and Cl^- levels as compared to G_2. Obtained results proved that foliar applications of *S. platensis* at 100 mg/l have a high potential for improving growth, photosynthetic capacity, yield production, decreased ROS-induced oxidative damage, and reducing DNA damage in salt-stressed *Phaseolus vulgaris*.

Keywords: Cyanobacteria, Macronutrient content, Photosynthetic efficiency ($^{14}CO_2$ fixation), *S. platensis*, Transpiration rate.

Introduction

One of the most important grain legumes for human nutrition and of significant commercial value is *Phaseolus vulgaris* L. (Moussa & Hassen, 2018). Beans are regarded as the second- and fourth-largest sources of protein in tropical America and eastern and southern Africa, respectively (Cardona & Kornegay, 1990). Salinity is a significant stressor that restricts plant growth and development by changing their morphological, physiological, and biochemical characteristics (Moussa & Hassen, 2018; Zhang et al., 2018). Salinity increases respiration rate and ion toxicity and decreases photosynthetic rate, protein synthesis, and enzymatic reactions (Moussa, 2006; Ahanger et al., 2020). Salinity induces the overproduction of reactive oxygen species (ROS) and H_2O_2. Damage from the ROS occurs to proteins, DNA, and lipids, causing membrane lipid peroxidation

#Corresponding author email: helal_moussa@hotmail.com ORCID: https://orcid.org/0000-0002-0041-7573
Received 10/10/2022; Accepted 05/03/2023
DOI: 10.21608/ejbo.2023.168006.2165
Edited by: Prof. Dr. Ahmed M. Saleh, Faculty of Science at Yanbu, Taibah University, KSA.

(malondialdehyde) and chlorophyll degradation (Yildirim et al., 2008; Kohli et al., 2019; Mansoor et al., 2022). Accumulation of proline is a common metabolic adjustment to maintain water relation in plant tissue under salinity (Saha et al., 2015). The MDA is routinely used as an index of lipid peroxidation under stress conditions (Mostofa et al., 2014). High levels of salinity are associated with a reduction of protein synthesis, increased Na^+ accumulation in plant organs, inhibition of enzymatic reactions, plant development, and decreased productivity (Tester & Davenport, 2003; Ahammed et al., 2018; Khan et al., 2020). To avoid using expensive synthetic fertilizers that cause water and soil pollution, cyanobacteria species were suggested as biofertilizers (Sánchez et al., 2003). The blue-green microalga *Spirulina platensis* extracts increased seed germination, plant growth, flowering, and fruit production by enhancing mineral nutrient utilization (Tuhy et al., 2015). The S. *platensis* biomass has a high concentration of γ-Linolenic acid (24.45g/100g), protein (47%), calcium (207mg/100g), iron (16.27mg/100g), and potassium (1675mg/100g) content (Milena et al., 2021). *Spirulina platensis* treatments stimulate a variety of plant metabolic responses, including respiration, photosynthesis, nucleic acid synthesis, chlorophyll formation, and ion absorption (Górka et al., 2018). To enhance cell division, *Spirulina platensis* stimulates the creation of polyamine molecules (Katarzyna et al., 2012). The active components of algae act as physiologic activators because they may stimulate plant enzymes such as nitrate reductase and others involved in mineral absorption and transformation (Joubert & Lefranc, 2008). Cyanobacteria can reduce stressors in modern agriculture (Renuka et al., 2018). The blue-green micro alga, *Spirulina platensis* consists of different phytochemicals (protein, high amino acid, vitamin A, vitamin B-12, vitamin E, carotenoids, iron, phenolic acids, tocopherols, *β-Carotene*, amino acids, antioxidant, free radical scavenging compounds, polysaccharides, minerals, and fatty acids) of great medicinal applications including antiviral, anti-metastasis properties, immune stimulatory, anti-diabetic, cancer, tuberculosis and inflammation (Sariya et al., 2020). *Spirulina platensis* produces the sulfated polysaccharide calcium spirulan with antiviral activity against cytomegalovirus, measles, and herpes simplex viruses (Ayehunie et al., 1998).

The experiment's objective was to determine whether exogenous administration of foliar *S. platensis* (100mg/L) could reduce the detrimental effects of salinity on common beans. So, we study the effect of salinity with or without *S. platensis* treatments in common beans on some growth characteristics (shoot weight, plant height, number of leaves/plant, number of pods/plant, number of seeds/pod, pod weight, and 100-seed weights), as well as the content of carotenoids, chlorophyll *a*+*b*, photosynthetic activity (assimilation of $^{14}CO_2$), total protein content, antioxidant enzyme activities (CAT, SOD, GPX, and POD), malondialdehyde (MDA), free proline, transpiration rate, total phenol, and mineral contents (Na, Cl, N, P, and K). Furthermore, the importance of using *Spirulina platensis* in reducing DNA damage in salt-stressed *Phaseolus vulgaris* L. plants is being investigated.

Materials and Methods

Spirulina platensis biomass preparation

Spirulina platensis (Gomont) Geitler (strain MIYE 101) was acquired from the Phycology Lab, Faculty of Science, Zagazig University, Egypt. An inverted divert light microscope was used to identify the microalga up to species using the keys of (Prescott, 1984; Yamagishi, 1992; Vymazal, 1995). The width of the trichomes consists of cylindrical cells that are shorter than broad cells, with a diameter of 8 to 10µm and length of tens to hundreds of µm, and coiled cells with a diameter of 5-6µm. The *S. platensis* was cultivated on a standard Zarrouk culture medium (Zarrouk, 1966). Two-liter culture flasks were inoculated with 250mL of *S. platensis* culture, aerated with air pumps, and incubated at 25±3ºC with fluorescent light tubes at 50 $\mu Em^{-2}s^{-1}$. The pH was adjusted to be suitable for this *S. platensis* growth (9.0±0.2). The culture was supplied with an air pump (97% O_2 and 3% CO_2) to accelerate *S. platensis* growth. The biomass was harvested by centrifugation at 5000rpm for 15min. The cell pellets were cleaned four times and resuspended in sterile H_2O to remove traces of growth medium. The suspension was then centrifuged at 6000rpm for 20min. The collected biomass of *S. platensis* was dried in the air for 4 days, powdered by hand mortar, and stored at 5ºC until used.

Preliminary experiment

Two separate preliminary seed germination experiments were conducted to determine the appropriate concentrations of NaCl and *Spirulina platensis* for the main experiment.

Determination of NaCl concentrations

On the basis of acute LC50 values, the level of NaCl was calculated (data not included in the text). The germination test used five NaCl concentrations: 100, 150, 200, 250, and 300mM. 100 seeds of *Phaseolus vulgaris* were used, and they were distributed on double-layered filter papers (3mm, Whatman), which were then placed in 12cm Petri dishes and moistened with either 10mL of double-distilled water. Petri dishes were covered and incubated at 22±2°C. Once the seed coat was broken, the seeds were scored as having germinated. Inhibition of germination (%) was calculated in comparison to the control samples, and the LC50 values were calculated using methods described by Pokorska-Niewiada et al. (2018).

Estimation of optimum concentrations of Spirulina platensis

The result of the germination test was used to define the optimum concentrations of *Spirulina platensis* (data not included in the text). 100 seeds of *Phaseolus vulgaris* were germinated in different concentrations of *S. platensis* solution (0.0, 25, 50, 75, 100, 150, and 200mg/L). When the radicle reached 0.3 cm, the number of germinated seeds was recorded daily for seven days to calculate the germination index.

The germination index (GI) was calculated using the following equation:

$$GI = \frac{N_1}{1} + \frac{N_2}{2} + \frac{N_3}{3} + \cdots \frac{N_n}{n}$$

where N_1, N_2, N_3, …, N_n denotes the germinated seeds number on 1, 2, 3…..,n days.

Plant material and experimentation circumstances

The study was carried out in the green house of the Radioisotope Department, Atomic Energy Authority during October 2021 for a period of 85 days. Selected healthy seeds of *Phaseolus vulgaris* L. (cv. Giza 3) of equal size and same color were purchased from the Agricultural Research Centre in Egypt. Seeds were surface sterilised for three minutes with a 0.1% (w/v) solution of sodium dodecyl sulfate, and sterilised deionized water was used to properly wash the seeds; each pot contains eight seeds in three replicates. In standardized plastic containers, all sets of seeds were planted (35cm in depth and 40 cm in diameter), packed with 11 kg of sandy loam soil (2:1 w/w). According to Black (1965), a chemical and physical analysis of utilised soil was done prior to planting, resulting in 2.7% organic matter concentrations of accessible K, P, and N of 220, 100, and 180mg kg^{-1}, respectively. The pots were maintained in a controlled growth chamber with day and night temperature temperatures of 22 and 20°C, relative humidity (65-75%), and a photoperiod of 15 hours. The maximal density of photosynthetic photons in the growth chamber was (~440μM $m^{-2} \cdot s^{-1}$). After 10 days from seed emergence, the seedlings were thinned, leaving four identical seedlings in each pot to thrive. Each Pots were irrigated with 250mL every 5 days by full concentration of nutrient solution (Hoagland & Arnon, 1950). After the seedlings had emerged for 20 days, salt stress was applied with 200mM NaCl (20 $dS \cdot m^{-1}$). The current experiment was divided into four groups as follows: G_1 (control, H_2O), G_2 (200 mM NaCl)), G_3 *(S. platensis*, 100mg/L), and G_4 (200 mM NaCl + *S. platensis*, 100mg/L). Three times, after 7 days, 50 days, and 77 days from sowing (vegetative, flowering, and fruiting stages, respectively), a one-hand pressure sprayer filled with *S. platensis* suspension (100mg/L) with Tween-20 was used as a surfactant to sprays at a rate of 0.1% (v/v) to ensure maximum penetration to plant leaves. Early in the morning, spraying allowed for improved foliar penetration because the stomata were open. Pots were washed with 400 ml of distilled water once a week to prevent salt accumulation. Manual weed control was carried out when necessary. All chemical analysis, such as photosynthetic efficiency (fixation of $^{14}CO_2$), elemental estimation, enzyme assays, and the comet assay, were measured at the flowering stage.

Growth characteristics and features of plant yield

In addition, the fresh weight of pods/plant, 100 seed weights, plant height, seed number, pod number/plant, and pods weight (fresh and dry) at the time of harvest were recorded.

Chemical analysis

Chlorophyll *a*+*b* were measured according to Inskeep & Bloom (1985), and carotenoids were estimated by Kissimon (1999). The transpiration rate was measured as mentioned by Udayakumar et al. (1998). Protein content was determined using the Bradford method (Bradford, 1976), and proline was assessed by Bates & Tear (1973). The method Chen et al. (2013) recommended for measuring

malondialdehyde content was used. The method given by Sadasivam & Manickam (2008) was used to estimate the total phenol content.

Photosynthetic efficiency (fixation of $^{14}CO_2$)

According to Moussa (2008, 2009), measurements of photosynthetic efficiency were assessed at the Egyptian Atomic Energy Authority. $^{14}CO_2$ was produced inside the bell jar for the treatments by an interaction between 100 μCi $NaH^{14}CO_3$+100mg Na_2CO_3 and 15% HCl. A tungsten light was used to illuminate the samples (~350–400μM·m^{-2}·s^{-1}). Following a rapid detachment from the stem after 30 minutes, the leaves were weighed, frozen for five minutes to inhibit biological processes, and then extracted with 70% hot ethanol. The ^{14}C was assessed by a liquid scintillation counter (Nuclear Enterprises, LSC2-Scaler Ratemeter SR7, UK) as mentioned by Bray (1960).

Elemental estimation

Utilizing a flame photometer (Jenway model PFP-7, UK), Na^+, K^+, and Cl^- concentrations were measured in the dry matter of the shoot and root. To estimate phosphorus, the Prokopy (1995) approach was used; harvested plants (shoot and root) were dried in a forced-draft oven at 65-70°C. The concentration of phosphorus in root and shoot materials was determined using the molydovanado-phosphate method (yellow complex) after digestion in a mixture of concentrated nitric and perchloric acids (4:1). The colorimetric method was used for the determination of phosphorous concentrations in digest solutions. Briefly, by adding 3-ml digested solution, 2mL reagent, and 5mL deionized water. The absorbance reading was taken at 470nm. Kjeldahl's digestion method was used to determine the total nitrogen content of dry seed. Organic and mineral nitrogen are reduced to NH_4^+ in hot, concentrated sulfuric acid in the presence of a catalyst. The NH_4^+ is recovered by distillation or diffusion and estimated by titration or colorimetrically (Association of Official Analytical Chemists, 1995).

Enzymes assays

The Thomas et al. (1981) technique is utilized to calculate peroxidase (POD) activity. The POD activity was determined using 4-methylcatechol as a substrate. The increase in absorption caused by the oxidation of 4-methylcatechol by H_2O_2, was spectrophotometrically measured at 420nm. The reaction mixture contained 100mM sodium phosphate buffer (pH 7.0), 5mM 4-methylcatechol, 5mM H_2O_2 and 500μL of crude extract in a total volume of 3.0mL at room temperature. One unit of enzyme activity was defined as 0.001 change in absorbance per min, under assay conditions. Catalase (CAT) activity was measured spectrophotometrically at room temperature by monitoring the decrease in absorbance at 240nm resulting from the decomposition of H_2O_2. Catalase activity was measured according to the method of Chance & Maehly (1995). One unit (U) of catalase activity was defined as the amount of enzyme that caused an absorbance change of 0.001 per minute under assay conditions. The reaction mixture contained 100mM sodium phosphate buffer (pH 7.0), 30 mM H_2O_2 and 100μL of crude extract in a total volume of 3.0mL. Superoxide dismutase (SOD) activity was determined by measuring the inhibition in photoreduction of nitroblue tetrazolium (NBT) by SOD enzyme (Chance & Maehly, 1995). The reaction mixture contained 50mM sodium phosphate buffer (pH 7.6), 0.1mM EDTA, 50mM sodium carbonate, 12mM L-methionine, 50μM NBT, 10μM riboflavin and 100μL of crude extract in a final volume of 3.0mL. A control reaction was performed without crude extract. The SOD reaction was carried out by exposing the reaction mixture to white light for 15min at room temperature. After 15min incubation, absorbance was recorded at 560nm using a spectrophotometer. One unit (U) of SOD activity was defined as the amount of enzyme causing 50% inhibition of photochemical reduction of NBT. The Navrot et al. (2007) method was used to measure the glutathione peroxidase (GPX) activity. The GPX activity analysis is based on the fact that GPX can oxidize the reduced glutathione (GSH) to oxidized glutathione (GSSG) at the presence of H_2O_2. The generated GSSG is recycled to its reduced state, GSH, by glutathione reductase (GR) and NADPH to generate $NADP^+$. The GPX activity can be measured based on the decrease of NADPH (absorbance at 340 nm). One unit of GPX activity is defined as 1 μM of NADPH oxidized per minute at 37°C.

Comet assay

The Nandhakumar et al. (2011) procedure was used to detect DNA damage using the comet test. The assay was carried out following the preparation of two gel layers. The leaf tissues (50mg) were minced, suspended at 1mL/g in chilled homogenizing buffer (0.075M NaCl and

0.024M EDTA), and gently homogenized at 600g using a laboratory stirrer (REMI-RQ127A) for 2min. To obtain nuclei, the homogenate was centrifuged at 1000g for 10min at 0°C, and the precipitate was resuspended in 1mL chilled homogenizing buffer. 100µL of 2% regular melting point agarose (Genei, India) was quickly layered on a pre-cooled, fully frosted slide, and covered with a cover slip, and allowed to solidify. The nuclear preparation was mixed 1:1 (v/v) with 2% low melting point agarose (Genei, India). The cover slip was removed carefully, and a second layer of 100µL of the mixture was pipetted out on the slide, covered with the cover slip again, and allowed to gel at 4°C for 15min. The slide (without cover slip) was immersed in a chilled lysing solution (2.5M NaCl, 100mM EDTA, 10mM Tris–HCl,1% Sarkosyl,10% DMSO, and 1% Triton X-100, at pH 10) and kept at 4°C for 2h. The slides were then placed on a horizontal gel electrophoresis platform and covered with a chilled alkaline solution made up of 300mM NaOH and 1mm EDTA (pH 12.5). The slides were left in the solution in the dark for 15 min to allow unwinding of the DNA to occur, and then the DNA was electrophoresed at 4°C in the dark for 15min at 1 V/cm and approximately 250mA. The slides were gently rinsed in neutralization buffer (0.4 M Tris–HCl, pH 7.5) to neutralize the excess alkali. Each slide was stained with 50µL of 20µg/mL ethidium bromide and covered with a cover slip. The photomicrograph of each slide was taken in Leica Fluorescent Microscope (Model 300 FX) at the same magnification (40×).

Statistical evaluation

Results are examined using an analysis of variance (SPSS, version 10.0). The multiple range test by Duncan was utilised to distinguish between means.

Results

The data on growth characteristics of the *Phaseolus vulgaris*, plant height, leaf number/plant, and shoot weight (fresh and dry weight) have changed in response to various treatments, as shown in Table 1. The salinity treatment (G_2) significantly reduced plant height and leaf number per plant by 16.2 and 28.8 %, respectively, when compared to the control plant (G_1). In addition, they reduced shoot weight (fresh and dry weight) by 17.5% and 36.2%, respectively, when compared to the control plant (G_1). At the vegetative stage, the G_3 (*S. platensis*, 100mg/L) and G_4 (200mM NaCl + *S. platensis*, 100mg/L) treatments showed a significant increase in all growth characteristics (plant height, leaf number/plant, shoot weight) when compared to the G_2 treatment (200mM NaCl) or control group (G_1), as shown in Table 1.

The yield characteristics of *P. vulgaris* are listed in Table 2. The salinity treatment (G_2) decreased significantly the number of pods/plant, the number of seeds/pod, and the 100 seed weights by 34.8, 23.1, and 27.6%, respectively, as compared to the control plant (G_1). The G_4 treatment (200mM NaCl + *S. platensis*, 100mg/L) increased the number of pods per plant, seeds per pod, and 100 seed weights by 28.4, 32.2, and 25%, respectively, when compared to the G_2 application (200mM NaCl). The G_4 treatment (200mM NaCl+ *S. platensis*, 100mg/L) showed a significant increase in number of pods/plant, number of seeds/pod, and 100 seed weights by 28.4, 32.2, and 25%, respectively, as compared to the G_2 treatment (200mM NaCl). Also, they decreased pod weight (fresh and dry) by 13.8 and 32.9% as compared to the control plant (G_1). Meanwhile, they increased pod weight (fresh and dry) by 30.6 and 28.9% respectively, compared to G_2 treatment (200mM NaCl), as shown in Table 2.

TABLE 1. Changes in vegetative parameters of *Phaseolus vulgaris* L. plants cultivated with or without NaCl and foliar-applied *S. platensis*

Groups	Plant height (cm)	Number of leaves/plant	Shoot weight/plant (gm)	
			Fresh	Dry
G_1	27.8^b	7.3^b	29.2^c	5.9^c
G_2	23.3^c	5.2^c	24.1^d	3.7^d
G_3	29.7^a	9.8^a	36.3^a	6.2^a
G_4	28.6^a	8.6^a	33.6^b	5.4^b

- G_1 (Control, H_2O); G_2 (Salinity, 200mM NaCl); G_3 (*Spirulina platensis*, 100mg/L); and G_4 (Salinity, 200mM NaCl + *Spirulina platensis*, 100mg/L).
- Data represent are means of three replicates±SE.
- Different letters indicate significant differences ($P \leq 0.05$).

TABLE 2. Changes in yield characteristics of *Phaseolus vulgaris* L. plants cultivated with or without NaCl and foliar-applied *S. platensis*

Groups	Number of pods/ plant	Number of seeds/pod	Pod weight/plant (gm)		100 seed weights (gm)
			Fresh	Dry	
G1	5.00^{b}	3.42^{b}	3.18^{b}	0.88^{b}	65.5^{b}
G2	3.26^{d}	2.63^{c}	2.74^{c}	0.59^{c}	47.4^{c}
G3	6.53^{a}	5.50^{a}	4.56^{a}	0.97^{a}	70.9^{a}
G4	4.55^{c}	3.88^{b}	3.95^{b}	0.83^{b}	63.2^{b}

- G_1 (Control, H_2O); G_2 (Salinity, 200mM NaCl); G_3 (*Spirulina platensis*, 100mg/L); and G_4 (Salinity, 200mM NaCl + *Spirulina platensis*, 100mg/L).
- Data represent are means of three replicates±SE.
- Different letters indicate significant differences (P≤ 0.05).

The salinity treatment (G_2) decreased significantly the chlorophyll *a*+*b*, carotenoids, and photosynthetic activity by 34.3, 52.6, 29.4%, respectively, and on the contrary increased significantly the transpiration rate by 79.6% compared to the control plant (G_1) as shown in Figs. 1-3. However, G_4 application (200mM NaCl+ *S. platensis*, 100mg/L) showed a significant increase in the chlorophyll *a*+*b*, carotenoids, and photosynthetic activity and significant decrease in the transpiration rate by as compared to salt stressed plant (G_2). The transpiration rate was significantly reduced by the exogenous application of *S. platensis*, 100mg/L (G_3) in comparison with the G_2 treatment (salinity, 200mM NaCl).

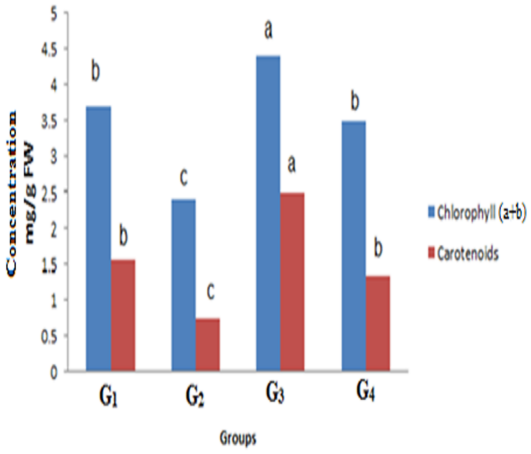

Fig. 1. Changes in chlorophyll *a*+*b*, and carotenoids in *Phaseolus vulgaris* L. plants cultivated with or without NaCl and foliar-applied *S. platensis* [Means within a column followed by the same letter (s) are not significantly different (P≤0.05)]

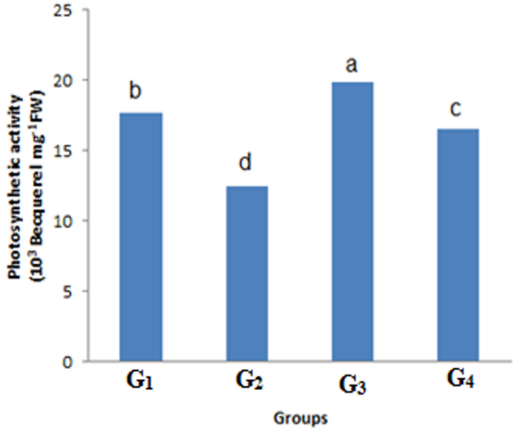

Fig. 2. Changes in photosynthetic efficiency in *Phaseolus vulgaris* L. plants cultivated with or without NaCl and foliar-applied *S. platensis* [Means within a column followed by the same letter (s) are not significantly different (P≤0.05)]

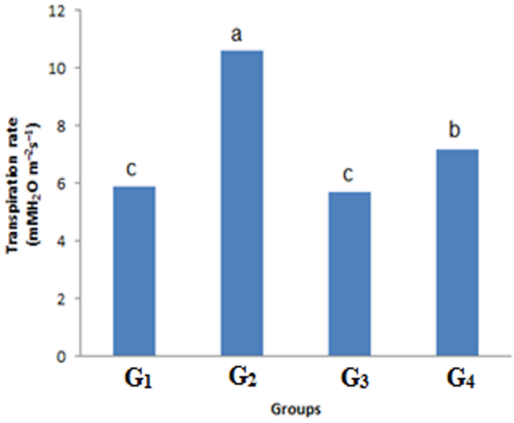

Fig. 3. Changes in the transpiration rate in *Phaseolus vulgaris* L. plants cultivated with or without NaCl and foliar-applied *S. platensis* [Means within a column followed by the same letter (s) are not significantly different (P≤0.05)]

Salinity treatment considerably increased the levels of SOD, POD, CAT, GPX, MDA, free proline, and total phenol by 24.3, 32.2, 45, 18.6, 56.5, 38.4, and 13.9%, respectively, as compared to the control plant (G_1). However, G_4 treatment (200mM NaCl + *S. platensis*, 100mg/L) showed a significant decrease in SOD, POD, CAT, GPX, MDA, free proline, and total phenol by 2.4, 4.5, 4.5, 9.5, 20, 9.4, and 2.7%, respectively, as compared to the control plant (G_1). Additionally, salinity significantly reduced total protein by 36% as compared to the control plant (Table 3). The G_4 treatment (200mM NaCl + *S. platensis*, 100mg/L), on the other hand, increased total protein content by 31.3% when compared to the salinity treatment (salinity, 200mM NaCl).

The salinity treatment (G_2) decreased significantly the macronutrient content of N, P, and K by 26.1, 34.7, and 43.7 %, respectively, and on the contrary, increased significantly the Na^+ and Cl^- levels by 55.8, 23.1 %, respectively, compared to the control plant (G_1), as shown in Fig 4. In contrast to salt-stressed plants, G_3 (*S. platensis*, 100 mg/l) and G_4 (200 mM NaCl + *S. platensis*, 100 mg/l) applications considerably enhanced the macronutrient content and reduced the Na^+ and Cl^- concentrations (Fig. 4).

Another frequent result of oxidative stress in plant tissues is the impact of reactive oxygen species on DNA damage. It is evident that under stress conditions that cause oxidative damage, plants experience some form of osmotic stress. The majority of macromolecules and nucleic acids are targets of salinity because it is a strong ROS in biological systems. Measuring the comet tail for a critical examination revealed 14-fold increases under salinity (200 mM NaCl), compared to the control (Fig. 5). Additionally, G_4 application (200 mM NaCl + *S. platensis*, 100 mg/l) reduced the comet tail by (50.6%) when compared to salinity treatment (G_2), serving as an alleviator to lower the rate of oxidative damage to nuclei (Fig. 4). Salinity typically causes the nuclear membrane to disintegrate through a peroxidation process, resulting in comet-shaped DNA molecules (Fig. 6). Figure 6 showed images from a comet assay for *Phaseolus vulgaris* cells. In images 1 (control) and 2 (*Spirulina platensis*, 100 mg/l), the majority of the cells are seen without comets. DNA maintained the circular arrangement of the typical nucleus despite being squashed firmly. In image 3 (salinity, 200 mM NaCl), the profile of the nuclear DNA was altered, and a fluorescent stripe could be seen emanating from the nucleus. Image 4 (salinity, 200 mM NaCl +*Spirulina platensis*, 100 mg/l) revealed reduced cell damage, and without comets (Fig. 6).

TABLE 3. Changes in SOD (units mg^{-1}protein), POD (units mg^{-1}protein), CAT (µMH_2O_2/min.gFW), GPX (µMNADPH/min.gFW), MDA (µM g^{-1} FW), free proline (µmol g^{-1} FW), total phenol (mg g^{-1} DW), and total protein (mg g^{-1} FW) in *Phaseolus vulgaris* L. plants cultivated with or without NaCl and foliar-applied *S. platensis*

Groups	SOD	POD	CAT	GPX	MDA	Free proline	Total phenol	Total protein
G_1	8.4^c	13.4^b	4.4^b	9.6^b	3.0^c	330^c	37^b	161^b
G_2	11.1^a	19.8^a	8.0^a	11.8^a	6.9^a	536^a	43^a	103^d
G_3	9.0^b	13.6^b	4.1^b	9.3^b	3.1^c	331^d	35^b	174^a
G_4	8.5^c	12.8^c	4.2^b	8.7^c	3.6^b	361^b	38^c	150^c

- G_1 (Control, H_2O); G_2 (Salinity, 200mM NaCl); G_3 (*Spirulina platensis*, 100mg/L); and G_4 (Salinity, 200mM NaCl + *Spirulina platensis*, 100mg/L).
- Data represent are means of three replicates±SE.
- Different letters indicate significant differences ($P \leq 0.05$).

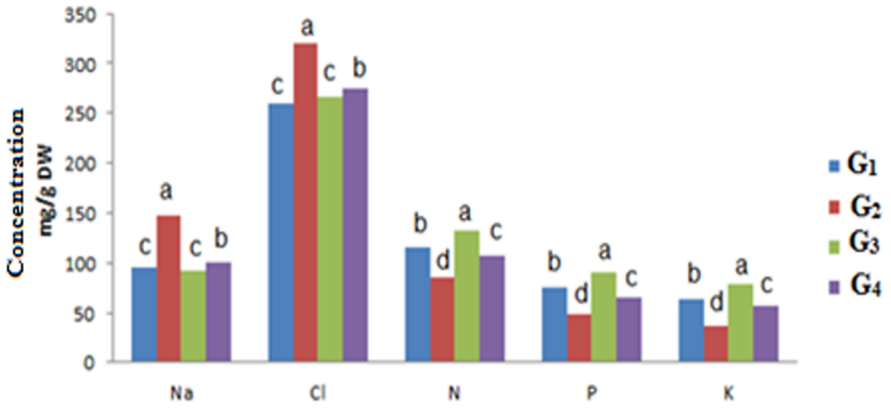

Fig. 4. Levels of Na, Cl, N, P, and K in root and shoot of *Phaseolus vulgaris* L. plants cultivated with or without NaCl and foliar-applied *Spirulina platensis* [Means within a column followed by the same letter (s) are not significantly different (P≤0.05)]

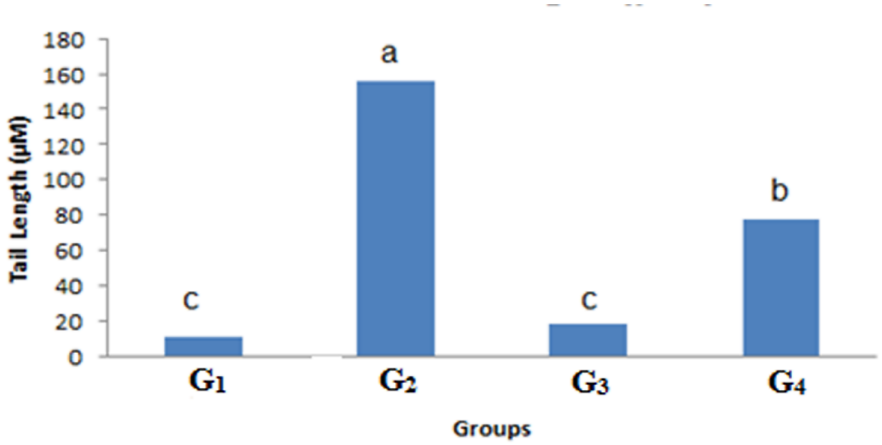

Fig. 5. Studying comet tail length-based nuclear damage in *Phaseolus vulgaris* L. plants cultivated with or without NaCl and foliar-applied *Spirulina platensis* [Means within a column followed by the same letter (s) are not significantly different (P≤0.05)]

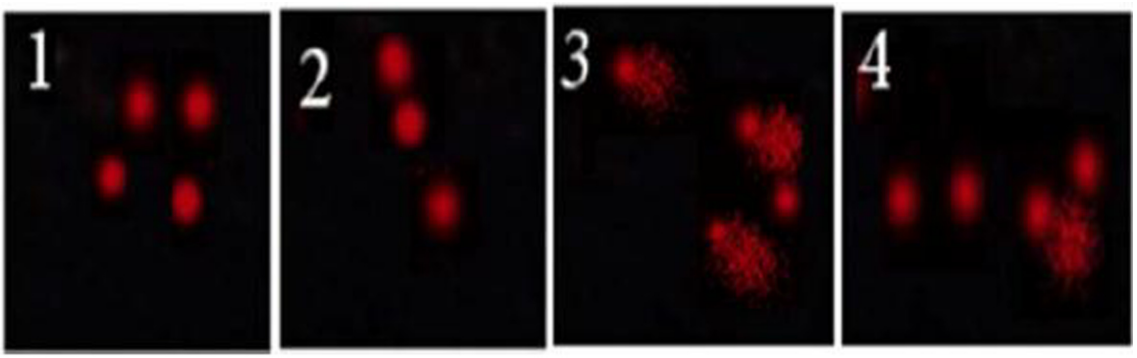

Fig. 6. Images from a comet assay for *Phaseolus vulgaris* L. cells. Images 1 (Control) and 2 (*Spirulina platensis*, 100mg/L), the majority of the cells are seen without comets. DNA maintained the circular arrangement of the typical nucleus despite being squashed firmly. Image 3 (Salinity, 200mM NaCl), the profile of the nuclear DNA was altered, and a fluorescent stripe could be seen emanating from the nucleus. Image 4 (Salinity, 200mM NaCl +*Spirulina platensis*, 100mg/L) revealed reduced cell damage, and without comets

Discussion

One or more of the following factors may have contributed to salinity (1) salt accumulation in the uppermost layer of the soil as a result of over-irrigation; (2) the sea is nearby; (4) capillary increase of salts in the root zone due to subterranean water as a result of overly rapid evaporation; (5) inadequate rainfall; (6) a greater rate of evaporation; and (6) inadequate management of water (Semida et al., 2014; Kusvuran et al., 2016). Soil salinization induces osmotic stress, which encourages the overproduction of ROS and is associated with great damage to DNA, total lipids, and proteins (Semida et al., 2016; Rady et al., 2018). Plants employ a number of methods, including osmotic adjustment, ion homeostasis, and strengthening the antioxidant defence system, to prevent harmful consequences (Xiong & Zhu, 2002; Moussa & Abd El-Aziz, 2008; Mohamed et al., 2021). Microalgae biostimulants (cyanobacteria) are now used on a global scale to produce high-yield, high-quality crops that are safe for human consumption. As was predicted, in the current study, salt-stressed *Phaseolus vulgaris* plants that received *S. platensis* (100mg/L) foliar application treatment considerably enhanced yield and growth parameters (Rady et al., 2018; Singh, 2014). The majority of cyanobacterial strains are good biofertilizers because they can fix atmospheric nitrogen (Ronga et al., 2019). Instead of applying only organic fertilizers, the application of enriched organic *Spirulina platensis* algae (biofertilizers) was beneficial in enhancing the total yield both qualitatively and quantitatively (Priyadarshani & Rath, 2012). Cyanobacteria are an important group of gram-negative photo autotrophic bacteria (significant nitrogen-fixing bacteria) in various agricultural soils (Vargas & Novelo, 2007). Additionally, have an ever-evolving photosynthetic mechanism that produces oxygen (Prabina et al., 2004), develop growth-promoting compounds that significantly increase pigment content, and increase the activities of nitrate reductase, and photosynthetic efficiency (Nanjappan-Karthikeyan et al., 2007). Cyanobacteria can assimilate N_2 and transform into an accessible type of ammonia (vitamins and hormones) needed for plant growth (Priyadarshani & Rath, 2012). *Spirulina platensis* is an abundant source of growth-promoting substances like organic acids, phytohormones, free amino acids, cytokinins, gibberellins, and auxins, which are vital for increasing plant growth and total yield (Singh, 2014; Battacharyya et al., 2015; Mógor et al., 2018). Salinity stress induces ion toxicity, drought, and nutrient imbalance by reductions in absorption and transportation (as Na^+ competes with K^+ for binding sites necessary for active cellular function), which finally inhibits many biological and physiological processes, which eventually lead to decreasing plant growth and yield (Moussa & Mohamed, 2016; Moussa & Hassen, 2018). Salinity may have detrimental impacts on photosynthetic activity due to its inhibition of rubisco activity, restriction of CO_2 uptake, increased pigment concentration, and great increase in the transpiration rate (Marschner, 1986; Moussa, 2009). Salinity stress decreased the synthesis of proline, which acts as a free radical scavenger, a stabilizer for the integrity of membranes, a carbon and nitrogen source for improving of plant growth, a quick recovery from stress, and a high content of important nutrient macromolecules (Jain et al., 2001). Salinity stress increases the influx of Na^+, which is very toxic to many enzymes, causes osmotic imbalance, membrane disorder, osmotic imbalance, reduction in cell division, decreased photosynthetic activity, growth, and cell metabolism (Singh & Dhar, 2010). Cyanobacteria produce naturally powerful molecules, including carbohydrates, vitamins, phenolics, polysaccharides, proteins, amino acids, and phytohormones, which may function well together to promote plant growth (Singh, 2014). Treatment of the soil with *Spirulina platensis* increased micro- and macro-elements for the cultivated plants (Bhowmik et al., 2010). Cyanobacterial treatment improves soil physicochemical characteristics such as mineral contents and water-holding capacity (Singh et al., 2016; Selem, 2021). *Spirulina platensis* has the ability to mobilize insoluble inorganic phosphate forms, decrease Na^+ levels, increase soil fertility by increasing K^+ and N accumulation, cause strong bioaccumulation of many elements due to its high binding ability, and synthesise salt-stress proteins. Thus, it can overcome the problems of harmful salinity in plants (Zheng & Gao, 2008; Rady et al., 2013). Salinity increased H_2O_2 contents in the plant tissues, which greatly induced damage to DNA (Rodriguez et al., 2011). *Spirulina platensis* has a high content of organic acids, phytohormones, free amino acids, cytokinins, gibberellins, auxins, elevated vitamins, hormones, unique important polysaccharides, and phycocyanin contents, that increased significantly the activity of the enzymes found in the cell nucleus, which eventually leads to reduced DNA oxidative damage, accelerate DNA repair (Bhat & Madyastha, 2001; Kaji et al.,

2002; Mohy El.Din, 2020). *Spirulina platensis* application stimulated the antioxidant enzyme activities, increased the synthesis of many shock proteins, increased the manufacturing of photoprotective substances like scytonemin and mycosporine-like amino acids, and stimulated DNA damage repair (El-Bassiouny et al., 2015; Selem, 2021).

Conclusion

In the world, the common bean is the most important legume for direct human consumption. Additionally, the use of mineral fertilizers in agricultural production has major detrimental effects on the land and contaminates groundwater. Additionally, it builds up in the food chain, creating hazards. Also, salinity severely reduced crop productivity. The aforementioned issues have many potential remedies, one of which is using blue green algae (cyanobacteria) like *S. platensis* to alleviate the problems. Foliar application of *S. platensis* (100mg/L) as eco-friendly biofertilizers and biostimulants improved growth, photosynthetic capacity, and total yield production in salt-stressed *Phaseolus vulgaris* plants while reducing DNA damage and ROS-induced oxidative damage. The promotion of growth by *S. platensis* could be attributed to the nutrients, bioactive molecules, and phytohormones that scavenge ROS and alleviate their adverse effects through regulating the metabolic processes in the plant. The findings showed that algal extracts have a high potential for use in modern horticulture and agriculture for reducing salinity hazards due to their high nutritional benefits, ease of use, a safe nature, and low cost.

Financial support and sponsorship: This research did not receive any specific grant from funding bodies in the public, commercial, or not-for profit sectors.

Availability of data and materials: All data generated or analyzed during this study are included in this published article.

Conflicts of interest: The authors state that there are no conflicts of interest to disclose.

Authors' contributions: Conceptualization, revision and supervision (Moussa, H.R.), methodology, and writing original draft preparation, formal analysis, and writing (Mohamed A. Taha), review, editing, methodology, and prepare manuscript for publishing (Eldessoky S. Dessoky).

Ethics approval: Not applicable.

References

Ahammed, G.J., Li, Y., Li, X., Han, W., Chen, S. (2018) Epigallocatechin-3-gallate alleviates salinity-retarded seed germination and oxidative stress in tomato. *Journal of Plant Growth Regulation,* **37**, 1349-56.

Ahanger, M.A., Aziz, U., Alsahli, A.A., Alyemeni, M.N., Ahmad, P. (2020) Influence of exogenous salicylic acid and nitric oxide on growth, photosynthesis, and ascorbate-glutathione cycle in salt stressed *Vigna angularis*. *Biomolecules,* **10**, 42.

Association of Official Analytical Chemists (1995) In: "*Official Methods of Analysis*". 16th ed. Association of Official Analytical Chemists, Arlington, Virginia.

Ayehunie, S., Belay, A., Baba, T.W., Ruprecht, R.M. (1998) Inhibition of HIV-1 replication by an aqueous extract of *Spirulina platensis* (*Arthrospira platensis*). *Journal of Acquired Immune Deficiency Syndromes*, **18**(1), 7-12.

Bates, L.S., Tear, L.D. (1973) Rapid determination of free proline for water-stress studies. *Plant and Soil*, **39**, 205-207.

Battacharyya, D., Babgohari, M.Z., Rathor, P., Prithiviraj, B. (2015) Seaweed extracts as biostimulants in horticulture. *Scientia Horticulturae*, **196**, 39-48.

Bhat, V.B., Madyastha, M. (2001) Scavenging of peroxynitrite by phycocyanin and phycocyanobilin from Spirulina platensis: protection against oxidative damage to DNA. *Biochemical and Biophysical Research Communications*, **285**, 262-266.

Bhowmik, D., Dubey, J., Mehra, S. (2010) Evaluating potential of Spirulina as inoculant for pulses. *Academic Journal of Plant Sciences*, **3**, 161-164.

Black, C. (1965) Method of soil analysis part 2. *Chemical and Microbiological Properties*, **9**, 1387-1388.

Bradford, M.M. (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein–dye binding.

Analytical Biochemistry, **72**, 248-254.

Bray, G.A. (1960) A simple efficient liquid scintillator for counting aqueous solutions in a liquid scintillation counter. *Analytical Biochemistry*, **1**, 276-285.

Cardona, C., Kornegay, J., Posso, C.E., Morales, F., Ramirez, H. (1990) Comparative value of four arcelin variants in the devel- opment of dry bean lines resistant to the Mexican bean weevil. *Entomologia Experimentalis et Applicata*, **56**, 197-206.

Chance, B., Maehly, A.C. (1995) Assays of catalases and peroxidases. *Methods in Enzymology*, **2**, 764-775.

Chen, P., Yan, K., Shao, H., Zhao, S. (2013) Physiological mechanisms for high salt tolerance in wild Soybean (*Glycine soja*) from Yellow River delta, China: photosynthesis, osmotic regulation, ion flux and antioxidant capacity. *PLoS ONE*, **8**, e83227.

El-Bassiouny, H.M.S., Abdallah, M.M.S., Rady, M.M., Gaballah, M.S., El-Sebai, T.N. (2015) Role of blue-green algae, glutathione and salicylic acid on the oxidative defense systems of wheat plant grown in saline soil. *International Journal of Pharmtech Research,* **8**(10), 18-31.

Górka, B., Korzeniowska, K., Lipok, J., Wieczorek, P.P. (2018) The Biomass of algae and algal extracts in agricultural production. In: "*Algae Biomass: Characteristics and Applications*"; Springer Science and Business Media LLC: Berlin/Heildeberg, Germany, 2018; pp. 103-114.

Hoagland, D.R., Arnon, D.I. (1950) The water culture method for growing plants without soil. *California Agricultural Experiment Station Circular*, **347**, 1-32.

Inskeep, W.P., Bloom, P.R. (1985) Extinction coefficients of chlorophyll a and b in N, Ndimethylformamide and 80% acetone. *Plant Physiology*, **77**, 483-485.

Jain, M., Mathur, G., Koul, S., Sarin, N.B. (2001) Ameliorative effects of proline on salt stress induced lipid peroxidation in cell lines of groundnut (*Arachis hypogea* L.). *Plant Cell Reports*, **20**, 463-468.

Joubert, J.M., Lefranc, G. (2008) Seaweed phytostimulants in agriculture: recent studies on mode of action two types of products from algae: growth and nutrition stimulants and stimulants of plant defence reactions. Book of abstracts: Biostimulators in modern agriculture. Warsaw, 7–8 February, 2008, 16.

Kaji, T., Fujiwara, Y., Inomata, Y. (2002) Repair of wounded monolayers of cultured bovine aortic endothelial cells is inhibited by calcium spirulina, a novel sulfated polysaccharide isolated from *Spirulina platensis*. *Life Sciences*, **70**, 1841-1848.

Katarzyna, C., Agnieszka, S., Izabela, M. (2012) The possibilities of the application of algal biomass in the agriculture. *Chemik*, **66**(11), 1235-1248.

Khan, I., Raza, M.A., Awan, S.A., Shah, G.A., Rizwan, M., Ali, B., Tariq, R., et al. (2020) Amelioration of salt induced toxicity in pearl millet by seed priming with silver nanoparticles (AgNPs): The oxidative damage, antioxidant enzymes and ions uptake are major determinants of salt tolerant capacity. *Plant Physiology and Biochemistry,* **156**, 221-232.

Kissimon, J. (1999) Analysis of the photosynthetic pigment composition. *International workshop and training course on microalgal biology and biotechnology. Mosonmagyarouar,* Hungary, pp. 13-26.

Kohli, S.K., Khanna, K., Bhardwaj, R., Allah, E.F., Ahmad, P., Corpas, F.C. (2019) Assessment of subcellular ROS and NO metabolism in higher plants: multifunctional signaling molecules. *Antioxidants*, **8**, 641.

Kusvuran, S., Kıran, S., Ellialtıoğlu, Ş.Ş. (2016) Antioxidant enzyme activities and abiotic stress tolerance relationship in vegetable crops. In: "*Abiotic and Biotic Stress in Plants- Recent Advances and Future Perspectives*", Arun Shanker, K., Shanker, Chitra (Eds.), pp. 481-506. Publisher: Intech, (Chapter: Chapter 21).

Mansoor, S., Wani, O.A., Lone, J.K., Manhas, S., Kour, N., Alam, P., Ahmad, A., Ahmad, P. (2022) Reactive oxygen species in plants: from source to sink. *Antioxidants*, **2022**, 11, 225.

Marschner, H. (1986) "*Mineral Nutrition in Higher Plants*". Academic Press, London.

Milena, M.R., Carolina, E.B., Jorge, M.O., Alexa, P.A., Diana, K.B.A. (2021) *Spirulina platensis* Protein as Sustainable Ingredient for Nutritional Food Products Development. *Sustainability*, **13**, 6849, 2-18.

Mógor, Á.F., Ördög, V., Lima, G.P.P., Molnár, Z.,

Mógor, G. (2018) Biostimulant properties of cyanobacterial hydrolysate related to polyamines. *Journal of Applied Phycology*, **30**, 453-460.

Mohamed, H., Moussa, H., Rgab, M. (2021) Does Exogenous Application of Melatonin Ameliorate Lead Toxicity in Eruca vesicaria Plants?. *Egyptian Journal of Botany*, **61**(1), 33-40.

Mohy El.Din, Soad, M. (2020) Evaluation of Different Biological Activities of *Spirulina platensis* Extracts. *Egyptian Journal of Botany,* **60**(1), 249-259.

Mostofa, M.G., Yoshida, N., Fujita, M. (2014) Spermidine pretreatment enhances heat tolerance in rice seedlings through modulating antioxidative and glyoxalase systems. *Plant Growth Regulation*, **73**, 31-44.

Moussa, H.R. (2006) Influence of exogenous application of silicon on physiological response of salt-stressed maize (*Zea mays* L.). *International Journal of Agriculture and Biology*, **2**(8), 293-297.

Moussa, H.R. (2008) Gamma irradiation effects on antioxidant enzymes and G_6PDH activities in *Vicia faba* plants. *Journal of New Seeds,* **9**(1), 89-99.

Moussa, H.R. (2009) Irradiation and aging effects on germination and seedling development of Garlic. *International Journal of Vegetable Science*, **15**, 240-252.

Moussa, H.R., Abd El-Aziz, S.M. (2008) Comparative response of drought tolerant and drought sensitive maize genotypes to water stress. *Australian Journal of Crop Science*, **1**(1), 31-36.

Moussa, H.R., Mohamed, A.H. (2016) Growth enhancers to mitigate salinity stress in *Vicia faba*. *International Journal* of *Vegetable Science*, **22**(3), 243-250.

Moussa, H.R., Hassen, Amira M. (2018) Selenium Affects Physiological Responses of *Phaseolus vulgaris* in Response to Salt Level. *International Journal of Vegetable Science*, **24**(3), 236-253.

Nandhakumar, S., Parasuraman, S., Shanmugam, M.M., Ramachandra, R.K., Chand, P., Bhat, B.V. (2011) Evaluation of DNA damage using single-cell gel electrophoresis (Comet Assay). *Journal of Pharmacology and Pharmacotherapeutics*, **2**, 107-111.

Nanjappan-Karthikeyan, Radha-Prasanna, Lata-Nain, Kaushik, B.D. (2007) Evaluating the potential of plant growth promoting cyanobacteria as inoculants for wheat. *European Journal of Soil Biology,* **43**(1), 23-30.

Navrot, N., Rouhier, N., Gelhaye, E., Jacquot, J.P. (2007) Reactive oxygen species generation and antioxidant systems in plant mitochondria. *Physiologia Plantarum*, **129**(1), 185-195.

Pokorska-Niewiada, K., Rajkowska-Myśliwiec, M., Protasowicki, M. (2018) Acute lethal toxicity of heavy metals to the seeds of plants of high importance to humans. *Bulletin of Environmental Contamination and Toxicology,***101**, 222-228.

Prabina, B.J., Kumar, K., Kannaiyan, S. (2004) Growth pattern and chlorophyll content of the cyanobacterial strains for their utilization in the quality control of cyanobacterial biofertilizers. *Biofertilizers Technology*, 446-450.

Prescott, G.W. (1984) "*How to Know the Freshwater Algae*". 3rd ed. Wm. C. Brown Company Publication, Iowa, USA. 384p.

Priyadarshani, I., Rath, B. (2012) Commercial and Industrial Applications of Micro Algae – A Review. *Journal of Algal Biomass Utilization*, **3**(4), 89-100.

Prokopy, W.R. (1995) Phosphorus in acetic acid extracts. Quickchem Method 12-115-01-1-1- B. Lachat Instruments, Milwaukee, WI.

Rady, M.M., Varma, B.C., Howladar, S.M. (2013) Common bean (*Phaseolus vulgaris* L.) seedlings overcome NaCl stress as a result of presoaking in *Moringa oleifera* leaf extract. *Scientia Horticulturae*, **162**, 63-70.

Rady, M.M., Taha, S.S., Kusvuran, S. (2018) Integrative application of cyanobacteria and antioxidants improves common bean performance under saline conditions. *Scientia Horticulturae*, **233**, 61-69.

Renuka, N., Guldhe, A., Prasanna, R., Singh, P., Bux, F. (2018) Microalgae as multi-functional options in modern agriculture: Current trends, prospects and challenges. *Biotechnology Advances***, 36**, 1255-1273.

Rodriguez, E., Azevedo, R., Fernandes, P., Santos, C. (2011) Cr (VI) induces DNA damage, cell cycle

arrest and polyploidization: a flow cytometric and comet assay study in *Pisum sativum*. *Chemical Research in Toxicology*, **24**, 1040-1047.

Ronga, D., Biazzi, E., Parati, K., Carminati, D., Carminati, E., Tava, A. (2019) Microalgal biostimulants and biofertilisers in crop productions. *Agronomy*, **9**, 192.

Sadasivam, S., Manickam, A. (2008) "*Biochemical Methods*", 3rd ed. New Age International (P) Ltd. Publishers, New Delhi.

Saha, J., Brauer, E.K., Sengupta, A., Popescu, S.C., Gupta, K., Gupta, B. (2015) Polyamines as redox homeostasis regulators during salt stress in plants. *Frontiers in Environmental Science,* **3**(21), 1-13.

Sánchez, M., Bernal-Castillo, J., Rozo, C., Rodriguez, I. (2003) Spirulina (Arthrospira): an edible microorganism. A review. *Universitas Scientiarum*, **8**, 1. Bogotà, Colombia.

Sariya, M., Punnida, A., Pilaiporn, T., Warathit, S., Supachai, Y., Siri, C., Wirawit, P., Pornngarm, L. (2020) Photochemoprotective effects of *Spirulina platensis* extract against UVB irradiated human skin fibroblasts**.** *South African Journal of Botany*, **130**, 198-207**.**

Selem, Eman (2021) Physiological Effects of *Spirulina platensis* in Salt Stressed V*icia faba* L. Plants. *Egyptian Journal of Botany,* **59**(1), 185-194.

Semida, W.M., Taha, R.S., Abdelhamid, M.T., Rady, M.M. (2014) Foliar-applied α-tocopherol enhances salt-tolerance in *Vicia faba* L. plants grown under saline conditions. *South African Journal of Botany,* **95**, 24-31.

Semida, W.M., Abd El-Mageed, T.A., Howladar, S.M., Rady, M.M. (2016) Foliar-applied α- tocopherol enhances salt-tolerance in onion plants by improving antioxidant defense system, *Australian Journal of Crop Science,* **10**(7), 1835-2707.

Singh, J.S., Kumar, A., Rai, A.N., Singh, D.P. (2016) Cyanobacteria: a precious bio-resource in agriculture, ecosystem, and environmental sustainability. *Frontiers in Microbiology,* **7**(529), 1-19.

Singh, N.K., Dhar, D.W. (2010) Cyanobacterial reclamation of salt-affected soil. In: "*Genetic Engineering, Biofertilisation, Soil Quality and Organic Farming*". Springer, Netherlands, pp. 243-275.

Singh, S. (2014) A review on possible elicitor molecules of cyanobacteria: Their role in improving plant growth and providing tolerance against biotic or abiotic stress. *Journal of Applied Microbiology,* **117**, 1221-1244.

Tester, M., Davenport, R. (2003) Na^+ tolerance and Na^+ transport in higher plants. *Annals of Botany*, **91**, 503-507.

Thomas, R.L., Jen, J.J., Morr, C.V. (1981) Changes in soluble and bound peroxidase, IAA oxidase during tomato fruit development. *Journal of Food Science,* **47**, 158-161.

Tuhy, L., Smoraj, M., Witkowska, Z., Chojnacka, K. (2015) Biofortification of maize with micronutrients by Spirulina. *Open Chemistry*, **13**, 1119-1126.

Udayakumar, M., Rao, R.C.N., Wright, G.C., Ramaswamy, G.C., Ashok, R.S., Gangadhar, G.C., Hussain, I.S.A. (1998) Measurement of transpiration efficiency in field conditions. *Plant Physiology and Biochemistry*, **1**, 69-75.

Vargas, R., Novelo, E. (2007) Seasonal changes in periphyton nitrogen fixation in a protected tropical wetland. *Biology and Fertility* of *Soils*, **43**(3), 367-372.

Vymazal, J. (1995) "*Algae and Element Cycling in Wetlands*". CRC Press, Inc., Boca Raton, Florida, USA, 689p.

Xiong, L., Zhu, J.K. (2002) Molecular and genetic aspects of plant responses to osmotic stress, *Plant, Cell & Environment,* **25**, 131-139.

Yamagishi, T. (1992) "*Plankton Algae in Taiwan (Formosa)*". Uchida Rokakuku, Tokyo, Japan, 253p.

Yildirim, B., Yaser, F., Ozpay, T., TurkOzu, D., Terziolu, O., Tamkoc, A. (2008) Variations in response to salt stress among field pea genotypes (*Pisum sativum* sp. arvense L.). *Journal of Animal and Veterinary Advances*, **7**, 907-910.

Zarrouk, C. (1966) Contribution a L'etude D'une Cyanobacterie: Influence de Divers Facteurs

Physiques et Chimiques Sur la Croissance et la photosynthèse de *Spirulina Maxima* (Setchell et Gardner) Geitler. *Ph. D Thesis*, University of Paris, Paris, France, 84p. 1966.

Zhang, Y., Li, S., Liang, Y., Zhao, H., Hou, L., Shi, Y., Ahammed, G.J. (2018) Effects of exogenous spermidine and elevated CO_2 on physiological and biochemical changes in tomato plants under iso-osmotic salt stress. *Journal* of *Plant Growth Regulation*, **37**, 1222-1234.

Zheng, J., Gao, Y. (2008) Growth and calcium bioaccumulation of Spirulina in photoheterotrophic cultures. *Journal of Biotechnology*, **136**, 558-576.

تأثير الأسبيرولينا بلاتنسيس على الخصائص الفسيولوجية وتخفيف تلف الحمض النووي في نباتات الفاصوليا المجهدة ملحيا

محمد عبد المحسن طه[1]، هلال رجب موسى[2]، الدسوقى سالمان دسوقى[3،4]

[1]قسم بحوث البساتين- كلية الزراعة - جامعة المنوفية، المنوفية - مصر، [2]قسم النظائر المشعة - مركز البحوث النووية - هيئة الطاقة الذرية -الجيزة - مصر، [3]معهد بحوث الهندسة الوراثية الزراعية - مركز البحوث الزراعية -الجيزة - مصر، [4]قسم الأحياء - كلية العلوم - جامعة الطائف -الطائف - المملكة العربية السعودية.

ينخفض بشدة معدل إنتاج المحاصيل بسبب تأثير الملوحة الزائدة مما ينتج عنه ضعف في النمو وانخفاض معدل النشاط الضوئي وكذلك الإنتاجية ويصاحب ذلك اختلال التوازن الغذائي داخل النبات. كان الهدف من هذا البحث هو تحديد تأثير الرش الورقي بمستخلص طحلب الاسبيرولينا بلاتنسيس كمحفز للنمو، والمحصول، والخصائص الكيميائية وفي تقليل تأثيرات الملوحة على نبات الفاصوليا صنف جيزة 3. ولتحقيق هذا الهدف، أجريت تجربة أصص تحت ظروف الصوب الزراعية التابعة لقسم النظائر المشعة بهيئة الطاقة الذرية خلال شهر أغسطس من عام 2021. كانت معاملات التجربة كالتالي: معامله رقم 1 (كنترول بدون اي اضافات)، معاملة رقم 2 (200 مللي مول من كلوريد الصوديوم بما يعادل 20 ديسيمنز/متر)، معاملة رقم 3 (الرش بمستخلص الطحالب بمعدل 100 مليجرام /لتر) ، معاملة رقم 4 (200 مللي مول من كلوريد الصوديوم + الرش بمستخلص الطحالب بمعدل 100 مليجرام /لتر). تم الرش الورقي للنباتات بعد 15 و30 و50 يوم من الزراعة باستخدام مستخلص طحلب الاسبيرولينا بلاتنسيس بتركيز 100 مليجرام /لتر. تم اخذ القياسات الخضرية والخصائص الكيميائية في نهاية مرحلة النمو الخضري ومرحلة الاثمار. أوضحت النتائج ان النباتات المعاملة بتركيز 200 مللي مول من كلوريد الصوديوم بما يعادل 20 ديسيمنز/متر إلى انخفاض فى طول النبات، وعدد الأوراق، والوزن الرطب والجاف خلال مرحلة النمو الخضري، وعدد القرون لكل نبات، وعدد البذور/ القرون، والوزن الطازج والجاف للقرون أثناء الإثمار، ومعدل وزن 100بذرة، وايضا الكلوروفيل (أ + ب)، والكاروتينات، ونشاط التمثيل الضوئي (معدل تثبيت ثانى أكسيد الكربون المشع) والبروتين الكلي في بذور الفاصوليا. أدى تطبيق الرش الورقي بمستخلص طحلب الاسبيرولينا بلاتنسيس إلى تحسين الصفات محل الدراسة مقارنة بمعاملات الملوحة. اظهرت معاملة الملوحة الى وجود زيادة في SOD (سوبر أكسيد ديسموتاز)، POD (بيروكسيداز)، CAT (كاتلاز)، GPX (جلوتاثيون بيروكسيديز)، MDA (مالونديلديهيد)، البرولين الحر، معدل النتح والفينولات الكلية مقارنة بنباتات الكنترول. قللت المعاملة رقم اربعة (200 مللي مول من كلوريد الصوديوم + الرش بمستخلص الطحالب بمعدل 100 مليجرام /لتر) من الانزيمات السابق تقديرها مقارنه بالنباتات المعاملة بالملوحة فقط. ومع ذلك، أدت المعاملة بمستخلص طحلب الاسبيرولينا بلاتنسيس الي زيادة معنوية في محتوى العناصر الغذائية الكبرى وخفض محتوي الصوديوم والكلور مقارنة بالنباتات المعاملة بالملوحة فقط.