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Physiological Effects of *Spirulina platensis* in Salt Stressed V*icia faba* L. Plants

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SALINITY is one of the most important problems in Egypt. Soil salinity decreases growth, photosynthetic activity, and results in nutrient imbalance in plants. This study evaluated the efficacy of the foliar applied *Spirulina platensis* (100mg/L) in reducing salinity effects in *Vicia faba* L. plants. The treatments are, T_1 (control), T_2 (salinity, 135mM from NaCl, equivalent to 13dS m^{-1}), T_3 (*Spirulina platensis*, 100mg/L), and T_4 (salinity, 135mM$^+$ *Spirulina platensis*, 100mg/L). Salinity decreased significantly chlorophyll *a*+*b*, carotenoids, weights of 100 seed, photosynthetic activity ($^{14}CO_2$ fixation), transpiration rate , total protein and (N, P and K). Application of T_4 improved the above parameters as compared to salt-stressed plants.Salinity increased significantly the SOD, POD, CAT, GPX, MDA, free proline, total phenol and Na^+ and Cl^- as compared to the control plants. However, T_4 treatment decreased the activity of the above parameters as compared to salt-stressed plants. Foliar applied *Spirulina platensis* ameliorated adverse effects of salinity by enhancing total protein level, N, P and K and photosynthetic activity ($^{14}CO_2$ assimilation). This first attempt to evaluate the potential of *Spirulina platensis* as growth enhancer under salt-stressed *Vicia faba* indicated that exogenously applied S*pirulina platensis* (100mg/L) provided more benefit against salinity stress.

Keywords: *Vicia faba* L., Photosynthetic activity ($^{14}CO_2$ fixation), *Spirulina platensis*, Salt stress.

Introduction

Faba bean (*Vicia faba* L.) is one of the most important crops used for food, animal feed and industry (Moussa, 2008).

Salinity in the soil causes limitation in the plant growth, productivity particularly in arid and semi-arid areas (Shannon, 1998). Additionally, salinity leads to increase in respiratory rate, ion toxicity, alteration of plant growth, imbalance in the distribution of elements, instability of membranes as a result of replacing the sodium element instead of calcium (Marschner, 1986). However, salinity decreases the photosynthetic rate (Moussa et al., 2016). It is well known that the accumulation of sodium ions in leaf tissues causes a decrease in the enzymatic activities and protein synthesis which is strongly related to high concentrations of salinity (Tester & Davenport, 2003). The external addition of some plant growth regulators, osmoprotectants and fertilizers reduces the damages resulting from salt stress (Ashraf et al., 2008).Successful attempts used algae as a biofertilizer in the process of soil reclamation, such as alkali and calcareous soil (Hegde et al., 1999).

Spirulina platensis is one of the photosynthetic blue-green microalga. Nowadays it is produced commercially as a food source, animal feed and bio-fertilizer with high nutritional value (Sànchez et al., 2003). *S. platensis* has been used as biofertilizer for many crops in different application methods individually or combined with other organic fertilizers (Aly et al., 2008). Ali & Mostafa (2009) tested the effect of spraying or using soil application methods of potassium-humate and *S. platensis*, either individually or in combination and used both as a bio- organic fertilizer on sesame plant. They found that the combined foliar application showed the highest rates in plant height, number of capsules per plant, number of branches per plant, seed weight per plant and weight of one thousand seeds. While, combined soil application showed the highest yield and production of seeds and straw. Therefore, the use of cyanobacteria species as a biofertilizers has been recommended rather than the use of high-cost chemical ones. This is due to the fact that this chemical fertilizers cause pollution of soil and water. *Spirulina* biomass consists of about 62% amino acids and it contains also the whole spectrum of mixed natural carotene and xanthophyl phytopigments which

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are considered as a rich natural source of vitamin B-12 and antioxidants (Kemka et al., 2007). The cyanbacterium *Spirulina* is considered to be ideal dietary supplements and one of the best solutions for treating malnutrition problems in developing countries (Vendan & Rajeshwari, 1998)

The goal of this study was to demonstrate whether it is possible to use exogenous application of *S. platensis* to mitigate the harmful effects of salinity in faba bean plants.

Materials and Methods

Plant material and seedlings formation

Faba bean seeds cv. Giza 2 were purchased from the Crop Institute, Agriculture Research Center, Giza, Egypt. The seeds were surface sterilized by 0.1% (w/v) sodium dodecyl sulfate solution and rinsed with sterile deionized water. They were germinated in pots (40×35cm, height × diameter), filled with 10kg of a sandy loam soil with 2.5% organic matter and available N, P, and K concentrations of 170, 80 and 200mg kg^{-1}, respectively. Irrigation with 250ml of the Hoagland's nutrient solution (Hoagland & Arnon, 1950) was supplied to each pot every 5 days. Plants were grown in a controlled environment growth chamber with a 15h photoperiod; 65%–75% relative humidity; and day and night temperatures of 22 and 20°C. The maximum photosynthetic photon flux density at the plant canopy was ~440µM m^{-2} s^{-1}. Ten days after emergence plants were thinned to four seedlings per pot.

Salt stress experiment

The experimental design was a randomized complete block design with three replications. Salt stress was imposed at 20 days after seedling emergence.

Seedlings were irrigated with a full Hoagland solution containing either 0.0 (water-only control) or 135mM NaCl, equivalent to 13dS m^{-1}. The treatments are, T_1 (control), T_2 (salinity, 135mM), T_3 *(S. platensis*, 100mg/L), and T_4 (salinity, 135 NaCl mM + *Spirulina platensis*, 100mg/L). Pots were rinsed with 400ml of distilled water once weekly to avoid salt accumulation. The *S. platensis* (100mg/L) were added to Tween 20 to fix solutions to plant leaf surfaces. Solutions were applied once onto leaves in the morning at flowering stage (plants that are 50 days old) using a manual pump. All determinations were carried out at 65-day-old plant except, weights of 100 seed were determined at the harvest stage.

Chemical analysis

Pigment content was determined according to Inskeep & Bloom (1985). Transpiration rate was determined as described by Ludlow & Muchow (1990). Proline was determined according to Bates et al. (1973). Total protein content was quantified according to Bradford (1976). Malondialdehyde (MDA) content was determined as described by Chen et al. (2013). Total phenol content was estimated by the method described by Sadasivam & Manickam (2008).

Photosynthetic activity ($^{14}CO_2$ fixation)

Photosynthetic activity was measured in the Radioisotope Department, Atomic Energy Authority, Cairo, Egypt, according to Moussa (2008). One pot from each treatment was placed under a bell jar and $^{14}CO_2$ was generated inside the chamber due to a reaction between 10% HCl and 50µCi (1.87×106 Bq) $NaH^{14}CO_3$+100mg Na_2CO_3 as a carrier. The samples were illuminated with a tungsten lamp (~300–350µM m^{-2} s^{-1}). After 30min, leaves were quickly detached from the stem, weighed, and frozen for 5min to stop biochemical reactions and then subjected to extraction with 80% hot ethanol. The ^{14}C was assayed in ethanolic extracts using a Bray cocktail (Bray, 1960) and a liquid scintillation counter (LSC2-Scaler Ratemeter SR7, Nuclear Enterprises, Edinburgh, UK).

Elemental analysis

Determination of Na^+, K^+ and Cl^- was determined with a flame photometer (Jenway model PFP-7, Chelmsford, Essex, England). The method of Prokopy (1995) was used to estimate phosphorus. Total nitrogen was determined in dry seed using Kjeldahl method (AOAC, 1995).

Enzymes assays

Peroxidase activity (POD, EC 1.11.1.7) was estimated using the method of Thomas et al. (1981). Superoxide dismutase (SOD, EC 1.15.1.1) and catalase (CAT, EC 1.11.1.6) activities were determined as described by Chance & Maehly (1995). Glutathione peroxidase (GPX, EC 1.11.1.9) activity was determined as the decrease in absorbance at 340nm due to the oxidation of NADPH by the method of Navrot et al. (2007).

Statistical analysis

Data were analyzed using analysis of variance (SPSS, version 10.0, SPSS Inc., Chicago, Ill.). Means were separated with Duncan's multiple range tests.

Results

The presence of salinity decreased significantly chlorophyll contents (Chl *a*+*b*), carotenoids, weights of one hundred seeds, photosynthetic activity and transpiration rate in *Vicia faba* plants as shown in Table 1. Application of T_4 improved the above parameters as compared to plants grown under salinity.

The presence of salinity decreased significantly Chl *a*+*b*, carotenoids, weights of seeds, photosynthetic activity and transpiration rate in *Vicia faba* plants (Table 1). Application of T_4 improved the above parameters as compared to salt-stressed plants (Table 1).

The presence of salinity accumulated significantly the antioxidant enzymes (SOD, POD, CAT and GPX), MDA, free amino acid proline and total phenols. While, salinity minimized significantly the total protein content when compared to the non-stressed plants (Table 2). Meanwhile, foliar supplement with *S. platensis* in T_4 evoked a pronounced increase in the total protein content in comparison to salt stressed faba bean plants (Table 2). In addition, T_4 treatment decreased the activity of the antioxidant enzymes, MDA, free amino acid proline and total phenol as compared to salt stressed plants.

Concerning the elements contents, the presence of salinity increased significantly the sodium and chlorine ions as compared to T_1 and T_3. Meanwhile, T_4 treatment decreased significantly these parameters as compared to T_2. Salinity decreased significantly the macronutrients content (nitrogen, phosphorus and potassium) when compared to the control. However, T_4 treatment increased the macronutrient content as compared to T_2 (Table 3).

TABLE 1. Changes in Chl *a*+*b*, carotenoids, weight of one hundred seeds, photosynthetic activity and transpiration rate in *Vicia faba* plants grown with or without sodium chloride and foliar applied *S. platensis*.

Treatment	Chl (*a*+ *b*) (mg g^{-1} FW)	Carotenoids (mg g^{-1} FW)	Wt. of one hundred seeds (g)	Photosynthetic activity (10^3 Becquerel mg^1 FW)	Transpiration rate (mM H_2O m^{-2} s^{-1})
T_1	3.68^b	1.56^a	65.7^b	15.618^b	5.8^a
T_2	2.80^d	0.83^c	51.6^d	11.803^d	3.0^b
T_3	3.78^a	1.58^a	67.8^a	17.599^a	5.7^a
T_4	3.47^c	1.32^b	62.7^c	14.601^c	5.0^c

Data were expressed in triplicates. Data were analyzed statistically by Duncan's test. The different superscripts in the same column are significantly different (P<0.01).

T_1: Control, T_2: Salinity, T_3: *Spirulina platensis* and T_4: Salinity+*S. platensis*.

TABLE 2. Changes in SOD, POD, CAT, GPX, MDA, free amino acid proline, total phenols and total protein in *Vicia faba* plants grown with or without NaCl and foliar applied *S. platensis*.

Treatment	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)	Total protein (mg g^{-1} FW)
T_1	7.5^c	11.5^b	3.3^b	8.7^b	2.1^c	310^c	28^b	152^b
T_2	10.0^a	17.9^a	7.1^a	10.9^a	5.8^a	517^a	33^a	95^d
T_3	8.1^b	11.7^b	3.2^b	8.4^b	2.0^c	308^d	28^b	168^a
T_4	7.4^c	10.9^c	3.0^c	7.9^c	2.7^b	348^b	29^c	142^c

Data were expressed in triplicates. Data were analyzed statistically by Duncan's test. The different superscripts in the same column are significantly different (P<0.01).

T_1: Control, T_2: Salinity, T_3: *Spirulina platensis* and T_4: Salinity+*S. platensis*.

TABLE 3. Contents of sodium, chloride, nitrogen, phosphorus and potassium in *Vicia faba* plants grown with or without NaCl and foliar applied *S. platensis*.

Treatment	Sodium	Chloride	Nitrogen	Phosphorus	Potassium
			(mg g^{-1} DW)		
T_1	85 [c]	250 [c]	108[a]	66[a]	55[a]
T_2	138 [a]	312 [a]	86[d]	40[d]	27[d]
T_3	82 [c]	257 [c]	104[b]	62[b]	51[b]
T_4	91[b]	265 [b]	100[c]	56[c]	46[c]

Data were expressed in triplicates. Data were analyzed statistically by Duncan's test. The different superscripts in the same column are significantly different (P<0.01).

T_1: Control, T_2: Salinity, T_3: *Spirulina platensis* and T_4: Salinity+*S. platensis*.

Discussion

The present study was carried out to evaluate the use of *Spirulina platensis* as a growth enhancer (bio-fertilizer) in the salt-stressed *Vicia faba* plants. Data showed that, there was a significant decrease in the growth and yield (represented by the weight of one hundred seeds) as a result of salinization. This result corroborates with that of Moussa et al. (2016) who reported that salinity stress led to the production of excess reactive oxygen species (ROS) which induced a defect in different physiological and biological processes and caused oxidative stress, lipid peroxidation and ion toxicity (Na^+ and Cl^-), hence it reflected on plant growth and yield. Salt-stress causes a lot of nutritional changes due to the decrease in the absorption and/or stem transportation. This may be a result of the competition between sodium and potassium ions on the binding locations for cell function processes (Moussa & Hassen, 2017). Such reports are similar to those of Prasanthi & Vishnuvardhan (2015) and Hemida et al. (2015).

As shown in the present study, considerable changes in the photosynthetic activity, pigments content (Chl *a*+*b*, carotenoids) and transpiration rate were recorded in salt-stressed bean plants. Such findings are in accord with those of Moussa et al. (2016), who stated that harmful effects of salinization on the photosynthetic activity are a result of the decline in Rubisco activity, pigment content, restriction of carbon dioxide assimilation and probably a minimized transpiration rate. Salinity raised the activity of the 4 assayed antioxidant enzymes (SOD, CAT, POD, GPX), MDA and total phenols in *V. faba* plants indicating that the plants grow under salt-stress exert an oxidative defense mechanism to balance the ROS resulted from salinity (Ashraf, 2009 and Kaye et al., 2011).

The recorded rise in the production of antioxidant enzymes and the antioxidant substances (carotenoids and phenols) under the effect of salinity may have a valuable role in scavenging the ROS produced as a result of stress condition (Moussa et al., 2016).

Data showed that there was an increase in the content of proline under salt stress condition which was in a harmony with the results of Jain et al. (2001) and Chen & Dickman (2005). These authors reported that proline is a carbon and nitrogen source produced by stressed-plants to be used for rapid recovery from stress and for growth since such amino acid plays as a membrane stabilizer and free radical scavenger. As shown in the results, the total protein content was negatively affected in salt-stressed *V. faba* plant. This result is in agreement with those of Merril (1990) and Mahboobe & Akbar (2013) who found that in many plant species grow under salinity, the protein content deceased as well as protein pattern changes recorded. Decreasing of protein under saline stress may be attributed to salinity that causes physiological disorders due to the elimination of K^+ ions by roots of plant where K^+ is the key element in the synthesis of protein. Also, decrease in potassium diminishes the growth and development of plants (Chen et al., 2007). An inhibition in protein synthesis will be continued at long exposure to stress (Caplan et al., 1990).

Regarding the effect of salinity on N, P and K contents, results showed a significant decrease in such macronutrients in the stressed *V. faba* plants. Similar results were recorded by Talei et al. (2012) who noticed a decrease in N, P, K^+, Ca^{+2}, $Mg^{+2}$and Mn content at high salinity levels.

At salt-stress conditions, high concentrations of ions compete with nutrients, especially potassium

ions, leading to a decrease in the levels of this element. With increased exposure to sodium chloride concentrations, the level of sodium ions and chlorine ions increases as well as decreases in the levels of calcium, magnesium and potassium ions in many plants occur (Khan et al., 2003).

Potassium plays a significant role in stimulating the different metabolic process within the plant cell as enzymes activity, photosynthesis, respiration, carbohydrate synthesis, chlorophyll synthesis, balancing the water amounts in plant leaves and regulating the mechanism of closing and opening of stomata as direct result of the stress resistance (El-Defan et al., 1999). As a result of the decrease in the availability of potassium by salinity because of the presence of large concentrations of sodium and chlorine ions in the soil solution, a lot of damage in the nutrition of the plant occurs and therefore the presence of sufficient amounts of potassium element is a necessity for the plant life.

There are several reasons for the decrease in the availability of potassium ions in the presence of sodium chloride, including the antagonism of potassium and sodium ions at the uptake sites in the roots of plants, sodium ions affect the process of transfer of potassium ions to the xylem in the plant or inhibit the process of absorption (Dorudi & Siadat, 1999; Al-Harbi, 1995 and Heidari & Jamshid, 2010).

It is clear that salinity works to reduce the accumulation of nitrogen in plants (Garg et al., 1993). This is due to the fact that chloride uptake increases and accumulates under salinity stress in parallel with a decrease in nitrate concentrations in plant stems (Garg & Gupta, 1997).

Concentration of the phosphorus element is associated with the photosynthetic rate of the plant, but a decrease in such element will decrease the conversion of fixed carbon to starch (Overlach et al., 1993). For this reason, decreasing phosphorus under the effect of salinity will reduce shoot growth. The relationship between phosphorus and salinity is very complex one and there is no clear mechanisms explain the increase, decrease or unchanged absorption rates of phosphorus in most plant species under salt stress conditions (Grattan & Grieve, 1992). However, it is probably both chloride and sulphate ions in the simulated salt solutions depressed the uptake of PO-34 by plants and its accumulation in fruits (Ullah et al., 1994).

The effect of *S. platensis* foliar spray on *V. faba* plant growth rate is positively evident not only in growing plants under normal conditions but also in the growing plants under salt stress conditions. However, significant differences were recorded between *V. faba* plants treated with *S. platensis* solution and non-treated ones when grown under salt stress condition. The results revealed that the growth and yield of *V. faba* plants treated with *S. platensis* solution were increased compared with each of the controls and those affected by salinity. Enriched organic fertilizers with biofertilizers especially *S. platensis* were beneficial in improving plant yield (quantitatively and qualitatively rather than application of organic fertilizers alone (Ahmed et al., 2011). This increase could be attributed to the nitrogenase and nitrate reductase activity of algae, its association with plant surface, peptides and amino acids produced in algae filtrate, and some other compounds that stimulate plant growth (Karthikeyan et al., 2007).

In addition, the available form of ammonia necessary for plant growth can be supplied by most cyanobacteria which can fix the atmospheric nitrogen into an available form of ammonia and thus can be used as bio-fertilizer (Vaishampayan et al., 2001).

In the present results, the foliar application of algal extract with the irrigation with NaCl resulted in a significant improvement in the pigments contents, photosynthetic activity and transpiration rate. This can be attributed to the presence of many growth-stimulating substances produced by cyanobacteria and their ability to form combinations with vascular and non-vascular plants, such substances increased pigment contents and photosynthetic activity (Karthikeyan et al., 2007). Moreover, the ability of *S. platensis* to stimulate plant growth could be explained by the nitrogen fixation ability of this microalga, beside its elevated content of vitamins and hormones (Priyadarshani & Rath, 2012).

There are several factors affecting the change in the activity of antioxidant enzymes, including the species, the development and metabolic status of the plant and yet the duration and strength of the stress affecting them (Joseph & Jini, 2010).

The results strongly suggest that the use of *Spirulina platensis* foliar spray has reduced the salt stress effect within the plant by changing its physiochemical properties. Thus, the presence of large levels of antioxidant enzymes under the influence of salinity was decreased by using *S. platensis* spray.

There are beneficial reports about the effects of micro-algae extracts on stressed cultivated plants, e.g., *Dunaliella salina* and *Phaeodactylum tricornutum* extracts mitigated salt stress during germination process of bell pepper (Guzmán-Murillo et al., 2013) due to a significant reduction in superoxide radical production and a lower lipid peroxidation. Water extract of micro-algae *Spirulina maxima* and *Chlorella ellipsoida* improve wheat tolerance to salinity and enhanced antioxidant capacity of the whole grains produced by micro-algal- treated plants (Abd El-Baky et al., 2010).

There is a strong correlation between the tolerance of salt stress and the presence of an effective antioxidant system within the plant and therefore resistance to salt stress is closely related to the efficiency of such antioxidant system (Raza et al., 2007). This is because it is known that plants grow under salt stress are trying to build a complex and effective antioxidant system as well as ROS-scavenging enzymes such as: SOD, CAT, APX, GPX, and GR (Alscher et al., 1997 and Apel & Hirt, 2004) to overcome the effects of salinity.

Abd El-Bakyet al. (2008) stated that algal extracts can maintain the activity of enzymes in general and antioxidant ones in particular and also can protect the plant from the impact of oxidative damage and deals directly with ROS. In addition, the authors found that algal extracts can contain bioactive components that act as growth regulators such as auxins and cytokinins, thus reducing the effect of salinity stress on the metabolic activity of wheat plant. They also revealed that there is a reduction in the effect of salinity on the wheat plants after exposure to the algal extracts and such decrease was a result of reducing the levels of sodium ions, while at the same time increases the amounts of photosynthetic pigments. In addition, they explained the existence of a relationship between the improvement by using the algal extracts in wheat plants exposed to salt stress and the increase in antioxidant defense capabilities, which include non-enzymatic and enzymatic antioxidant systems that led to a reduction in the oxidative damage of the biologically active molecules and retention of many physiological processes in wheat plant such as photosynthesis and productivity.

As recorded in the present results, application of *S. platensis* to salt-stressed plants caused an excess in macronutrient N, P and K. This may be correlated with the fact that Cyanobacteria increases soil fertility by increasing the levels of available N and P. This represents an economic point of view providing about 50% of the necessary doses of nitrogen, phosphorus and potassium (Singh & Bisoyi, 1989 and Mahmoud et al., 2007).

S. platensis is also attractive for its highly binding ability to many elements (Mosulishvili et al., 2002 and Zheng & Gao, 2008). In cells exposed to salt stress, an increase in potassium and/or calcium uptake occurs at the expense of sodium absorption, such phenomenon is necessary to maintain the level of potassium/sodium ratio high and thus maintain the life of cells under stress conditions (Ashraf, 2004 and Kavitha et al., 2012).

The external spraying of the plants with *S. platensis* reduced the effects of salinity in the form of reducing the levels of sodium ions and raising the levels of K^+, N and P. This resulted in maintaining a high level of K/Na ratio as compared to the same levels in growing plants under the conditions of salt stress which did not receive any external treatment.

Conclusion

Cyanobacteria (*S. platensis*) showed a good potential to improve the growth and yield of *V. faba* plants grown under salt stress by its valuable components that scavenge ROS and alleviate its adverse effects through regulating the metabolic processes in the plant. Therefore, it is recommended to use the foliar spray with *S. platensis* as biofertilizer for salt stressed plants.

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الآثار الفسيولوجية لطحلب الإسبيرولينابلاتينيسيس في نبات الفول المُعّرضه للإجهاد الملحى

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تُعتبرالملوحة واحدة من أهم المشاكل في مصر و العالم فمن المعروف أن ملوحة التربة تقلل من النمو و نشاط التمثيل الضوئي وتؤدى إلى اختلال توازن المغذيات في النباتات. قيمت هذه الدراسة قدرة رش أوراق نبات الفول بطحلب الإسبيروبينا بلاتينسيس (بتركيز 100 مليجرام / لتر) في تقليل تأثيرات الملوحة في نبات الفول. إشتملت الدراسه على أربع معاملات وهى T1 (الكنترول: نبات غير مُعامّل) و T2 (نبات مُعرّض للإجهاد الملحى بتركيز 135 مللي مولار من كلوريد الصوديوم) و T3 (نبات مُعامّل بطحلب الإسبيروبينا بلاتينسيس بتركيز100 مليجرام/ لتر) و T4 (نبات مُعرّض للإجهاد الملحى بتركيز 135 ملي مولار من كلوريد الصوديوم و مُعامّل بالإسبيروبينا بلاتينسيس بتركيز 100 مليجرام/لتر). أدى نمو نبات الفول تحت الإجهاد الملحى إلى إنخفاض ملحوظ فى معدلات (الكلوروفيل *a* وكلوروفيل *b* و الكاروتينيدات) و وزن البذور و نشاط التمثيل الضوئي الُمتَمثل فى (تثبيت $^{14}CO_2$) و مُعدل النتح و مُعدل البروتين الكلى و مُعدلات النيتروجين و الفوسفور و البوتاسيوم. أدت المعامله T4 إلى تحسن فى الخصائص المذكورة مقارنةً بالمعامله T1. بالإضافه إلى ذلك أدى الإجهاد الملحى إلى زيادة ملحوظه فى نشاط الإنزيمات المضادة للأكسدة (سوبر أكسيد ديسميوتيز و بيروكسيديز و كاتاليز وجلوتاثيون بيروكسيديز) ومُعدل مالون داى الدهيد و البرولين الحر و الصوديوم و الكلور مقارنةً بالمعامله T1. على النقيض من هذة النتائج أدت المعامله T4 إلى إنخفاض ملحوظ فى مستويات القياسات السابق ذكرها مقارنه بالمعامله T2. أدى رش أوراق النبات بطحلب الإسبيروليناب لانتينيس إلى تخفيف آثار الإجهاد الملحى من خلال تحسين مستوى البروتين الكلي و النيتروجين و الفوسفور و البوتاسيوم ونشاط التمثيل الضوئي. تعتبر هذه الدراسه من الدراسات الأولى لتقييم إمكانات الرش الخارجى بطحلب الإسبيروبينا بلاتينسيس كمعزز للنمو لنبات الفول المُتّأثر بالإجهادالملحى.