Use of salicylic acid application and Azospirillum inoculation to improve wheat plant growth under saline conditions

*Saleh A. Khaled **Souhaib A. ElFraikh *** Rabha I. Mohamed

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Abstract: The experiment was conducted to evaluate *Azospirillum* inoculation or salicylic acid (1 m*M*) Spray the plant leaves during growth, nitrogen fixation and On some physiological characteristics under different NaCl concentrations (100, 200, 300 m*M*). The results are summarized as following : Wheat, *Triticum aestivum* L. tolerated NaCl salt stress up to 300 m*M*. Salt stress led to a decrease in some growth traits especially at high NaCl concentration (300m*M*). *Azospirillum* inoculation increased shoot and root length and leaf area by 59.0, 30.3cm / pot and 87.5Cm^2 / plant at 100 m*M* NaCl, and salicylic acid application recorded 59.0, 33.0 cm / pot and 77.8 Cm^2 / plantat100 m*M* NaCl compared with the control treatment, respectively. *Azospirillum* inoculation or salicylic acid application increased wheat dry shoot and root mass at different level of NaCl as compared with control plant. Bacterization increased chlorophyll a, b and Carotenoids contents by 2.46, 2.72 and 2.51mg/g at100 m*M* NaCl. In addition, salicylic acid application was recorded 2.94, 2.80 and 2.58 mg/gat a concentration of 100 m*M* of sodium chloride, respectively. *Azospirillum* inoculation or salicylic acid application highly significant increased shoot soluble carbohydrates at a concentration of 100 m*M* of sodium chloride. Wheat plant exposed to sodium chloride salt(200, 300 m*M*) exhibited a significant decreased in antioxidant enzymes activity like catalase and peroxidase activities. Whereas, addition of *Azospirillum* sp. or Salicylic to wheat plants with different salt levels significantly mitigated salt stress deleterious effect by increase antioxidant enzymes activity. When salt stress levels increase, it leads to an increase in hydrogen peroxide in the plant, while it was decreased by *Azospirillum* inoculation or salicylic acid application as compared with control plants *Azospirillum* inoculation was increased shoot and root total N-yield by 30.6 and 6.6 mg N/pot at 200 m*M* NaCl, respectively. The nitrogen derived atmosphere (%NdFa) by *Azospirillum* inoculation recorded 42.1% and 36.3% at 200 m*M* NaCl concentration and salicylic acid application recorded 33.3% and 20% in the shoot and root system at 100 mM NaCl concentration, respectively. Shoot minerals content (Ca, K, P) was changed by *Azospirillum* inoculation at 200 m*M* NaCl concentration compared with control. Results show that root minerals content of wheat plant was resisted salt stress levels by Salicylic acid application.

تحسين النمو وتحمل الملوحة لنبات القمح عن طريق تطبيق حمض الساليسيليك والتلقيح بالأزوسبيريلوم

*صالح عبد الرازق خالد قسم علم النبات –كلية العلوم – جامعة عمر المختار

** صهيب عبد العزيز الفريخ قسم علم النبات –كلية العلوم – جامعة درنة

*** رابحة أرحيم محمد المعهد العالي للعلوم والتقنية – شحات

المستخلص: أجريت تجارب في أصيص لتقييم الدور المحتمل للتلقيح بالأزوسبيريلوم أو الرش الورقي بحامض الساليسيليك (1 ملم) في النمو وتثبيت النيتروجين وبعض التغيرات الفسيولوجية للقمح المزروع تحت تراكيز مختلفة من كلوريد الصوديوم (100، 200، 300 ملم). وتتلخص النتائج فيما يلي: يتحمل القمح *Triticum aestivum* L. إجهاد ملح كلوريد الصوديوم حتى 300 ملم. أدى الإجهاد الملحي إلى انخفاض مؤشرات نمو

* **Botany Department. Faculty of Sciences, Omar Al-Mukhtar University saleh.khaled@omu.edu.ly**

** **Botany Department. Faculty of Sciences, Darna University**

*** **Higher Institute of Science and Technology – Shahat**

النبات (الطول، المساحة الورقية، الكتلة الطازجة والجافة) خاصة عند التركيز العالي من كلوريد الصوديوم (300 ملم). أدى التلقيح بالأزوسبيريلوم إلى زيادة طول المجموع الخضري والجذري ومساحة الورقة بمقدار 59.0، 30.3 سم/ أصيص و 87.5 سم^2 / نبات عند 100 ملي مول كلوريد الصوديوم، كما سجلت إضافة حامض الساليسيليك 59.0، 33.0 سم / أصيص و 77.8 سم^2 / نبات عند 100 ملي مول كلوريد الصوديوم مقارنة مع المعاملة القياسية، على التوالي. أدى التلقيح بالأزوسبيريلوم أو إضافة حامض الساليسيليك إلى زيادة المجموع الخضري الجاف وكتلة الجذور في القمح عند مستويات مختلفة من كلوريد الصوديوم مقارنة بنبات السيطرة. أدت عملية البكتريا إلى زيادة محتوى الكلوروفيل أ، ب والكاروتينويد بمقدار 2.46، 2.72 و2.51 ملجم/جم عند 100 ملي مولار كلوريد الصوديوم. بالإضافة إلى ذلك، تم تسجيل 2.94، 2.80 و 2.58 مليجرام/جرام من حمض الساليسيليك عند تركيز 100 ملي مولار من كلوريد الصوديوم مقارنة بالمعاملة القياسية، على التوالي. أدى التلقيح بالأزوسبيريلوم أو استخدام حامض الساليسيليك إلى زيادة معنوية كبيرة في إطلاق الكربوهيدرات القابلة للذوبان عند 100 ملي مول كلوريد الصوديوم مقارنة بمحطة التحكم. أظهرت براعم القمح وجذور النباتات المعرضة لكلوريد الصوديوم وخاصة (200، 300 ملم) انخفاضاً معنوياً في نشاط الإنزيمات المضادة للأكسدة مثل نشاط الكاتلاز والبيروكسيديز. حيث أن إضافة .Azospirillum sp أو الساليسيليك لنباتات القمح ذات المستويات الملحية المختلفة خفف بشكل كبير من التأثير الضار للإجهاد الملحي عن طريق زيادة نشاط الإنزيمات المضادة للأكسدة. زاد تراكم بيروكسيد الهيدروجين في نباتات القمح والجهاز الجذري لنباتات المكافحة عن طريق زيادة مستوى الإجهاد الملحي، في حين انخفض عن طريق التلقيح بالآزوسبيريلوم أو إضافة حامض الساليسيليك مقارنة مع نباتات المقارنة. 6.6 ملغم/N وعاء عند 200 ملي مول كلوريد الصوديوم، على التوالي. سجل تثبيت النيتروجين الجوي (NdFa%) عن طريق التلقيح بالأزوسبيريلوم 42.1% و36.3% عند تركيز 200 ملي مولار من كلوريد الصوديوم، كما سجل تطبيق حمض الساليسيليك 33.3% و20% في المجموع الخضري والجذري عند تركيز 100 ملي مولار من كلوريد الصوديوم، على التوالي. تم تغيير محتوى المعادن المطلقة (Ca، K، P) عن طريق التلقيح بالأزوسبيريلوم بتركيز 200 ملي مولار من كلوريد الصوديوم مقارنة مع التحكم. أظهرت النتائج أن محتوى جذور نبات القمح من المعادن قد تمت مقاومة مستويات الإجهاد الملحي عند إضافة حامض الساليسيليك.

INTRODUCTION:

The wheat plant belongs to the Poaceae family and is one of the strategic crops that is widely grown for its nutritional importance (Shewry, 2009).Wheat grains are rich in carbohydratesand proteins (Curtis *et al*. 2002, Shewry and Hey, 2015), Salinity affects the physiological and biological processes of plants(Yaycili and Alikamanoglu, 2012, James *et al*. 2011). Salt stress affects the productivity of food crops (Reguera*et al.* 2012, Soussana *et al.* 2012).Increased osmotic stress causes ionic toxicity(Rahnama*et al.* 2010; James *et al.* 2011).The negative effects of salinity are due to an increase in sodium and chlorine ions in the plant. Na+ and Cl- are the two main ions that cause many physiological problems in the plant. (Tavakkoli *et al.* 2010).When salt is stressed, the ability to absorb water by the roots decreases and the process of water loss from the leaves increases due to the effect of osmotic stress as a result of the increased concentration of high salts in the plant (Munns 2005).Salinity causes cell membrane damage, nutrient imbalance, changes in levels of growth regulators, inhibition of enzymes and photosynthesis, and protein and nucleic acid synthesis. (Mahajan and Tuteja 2005; Hasanuzzaman *et al.* 2012a; Apel and Hirt 2004; Turkan and Demiral 2009).Difficult plants with high salt concentration have an effect on cellular homeostasis, physiological and biochemical processes.Biochemical and molecular investigations of salt stress in plants have reported significant increases in reactive oxygen species . (Tanou *et al.* 2009; Ahmad *et al.* 2010, 2012).The ability of a plant to grow and complete its life cycle under stressful salt conditions (Yadav *et al*. 2011).In saline areas, limited by nitrogen deficiency. Applying nitrogen fertilizer to plants growing in dry climates may increase tolerance (Van-Hoorn *et al*., 2000).The increasing costs of nitrogen fertilizers and the risk of increased soil salinity will likely further limit nitrogen use in fields (Mohammed *et al*., 1989).Inoculation of crop seeds and seedlings with various plant growth-promoting bacteria (PGPB), such as Rhizobium and Azospirillum spp. Also, with mycorrhizal fungi, it reduces salt stress (Zahran, 1991; Ribaudo *et al*., 2001; El-Komy *et al*., 2004).*Azospirillum* has gained the reputation of being the most studied plant nitrogen-fixing rhizobacteria (Bashan and Holguin, 1997 ; Steenhoudt and Vanderleyden,2000).Salt stress enhances the performance of Azospirillum in field conditions with conditions that are not ideal for plant growth (Jofre *et al*., 1998 ; Reinhold *et al*., 1987 ; Hartmann and Baldani, 2006).There are many modes of action, such as N2 fixation, bioactive effects, and stimulation of nitrate reductase activity, which are sure to increase nitrate accumulation. (Boddey and Dobereiner.,1988)., Abdel Samad and Al-Komy (1998) and Al-Komy et al. 2003 and 2004), studied the potential role played by atmospheric nitrogen-fixing microorganisms under salt and drought stress on plants. The hormone (salicylic acid) plays an important role in regulating a number of physiological processes (Raskin, 1992; Hayat *et al*., 2007) and also has a major role during plant growth under salty conditions (Kaya *et al*., 2007). High salinity leads to disturbances in the cellular redox system in favor of oxidized forms and thus leads to DNA damage and enzyme inactivation (Smirnov, 1993). The ameliorative effects of SA are well recorded to work to stimulate plants to tolerate salinity (Aldesuquy *et al*., 1998, El-Tayeb, 2005; Gunes *et al*., 2005).Global production is being affected by increased salinity in arable land in dry and semi-arid lands (Sahi *et al*., 2006 ; Joseph et al., 2010 ; Sakhabutdinova *et al*., 2003).World interest in the ability of SA to exert a protective effect on plants under the influence of various stress factors has increased. Thus, the objectives of this study were designed to obtain information regarding the possible role of *Azospirillum* inoculation or Salicylic acid foliar application of wheat plants grown under salt-stress in greenhouse pot experiments in improving Salinity tolerance.

MATERIALS AND RESEARCH METHODS:

This research was conducted during the 2013-2014 growing season. To study the effect of different levels of Stalin (0.100, 200, 300 mmol sodium chloride) on the wheat plant, as well as the interactive effect of *Azospirillum* sp., foliar spraying with salicylic acid, and levels of Stalin on the growth and physiological processes of the plants.Wheat seeds,local variety from the Agricultural Research Center, Al-Fattayah - Libya. Seeds were selected and surface sterilized with a mixture of ethanol (90%) and H^2O^2 (25%) in a 1:1 (V:V) ratio for 3 minutes, followed by several washes with sterile distilled water.

Wheat seeds were planted in pots filled with field soil, placing five seeds in them, then adding the treatments to them. The pots were divided into three groups, each pot containing three seedlings 12 cm tall: the seedlings of the first group of pots were considered standard treatment, and the pots of the second group. The plants of the second group were inoculated with Azospirillum sp. The third group of pots and seedlings (two weeks old) were sprayed 3 times with 1 mm salicylic acid (25 ml per pot). The pots were adjusted to the Stalin level (100, 200, 300 mM NaCl).Supplemental irrigation was completed for all pot groups to maintain the field capacity necessary for seedling growth. The experiment followed a completely randomized design with three replications for each treatment (Figure 1).

Fig. 1: Wheat plants grown in a pot experiment at different Stalinization levels

CHARACTERISTICS UNDER STUDY:

Leaf area was calculated according to Norman and Campbell (1994).

Estimation of photosynthetic pigment contents pigment content (Chlorophyll *a* , Chlorophyll *b* , Carotenoids)was calculated according to Lichtenthaler and Wellburn (1983):

Extraction and estimation of soluble carbohydrate was calculated according toFales (1951) .

Enzymes were extracted and examined according to (Esfandiari*et al*. 2007b ; Yingsanga*et al.* 2008 ; Ranieri e*t al*. 1995) .

Hydrogen peroxide levels were determined according to the method of Sergiev*et al*. (1997).

nitrogen content was estimated spectrophoto-metrically at 420 nm using the Nessler's method as described by Hesse, (1971). The percentage of crude protein in plant tissues was found by multiplying the total nitrogen content (%N) by the factor 6.25 according to the Association of Official Agricultural Chemistry (A.O.A.C 1995).

Total nitrogen yield and fixed nitrogen according to the Rennie 1980 equation.

Determination of phosphorus (P), calcium (Ca), potassium (K), were according to Lachica*et al*.1973 ; Brown and Lillel 1946.

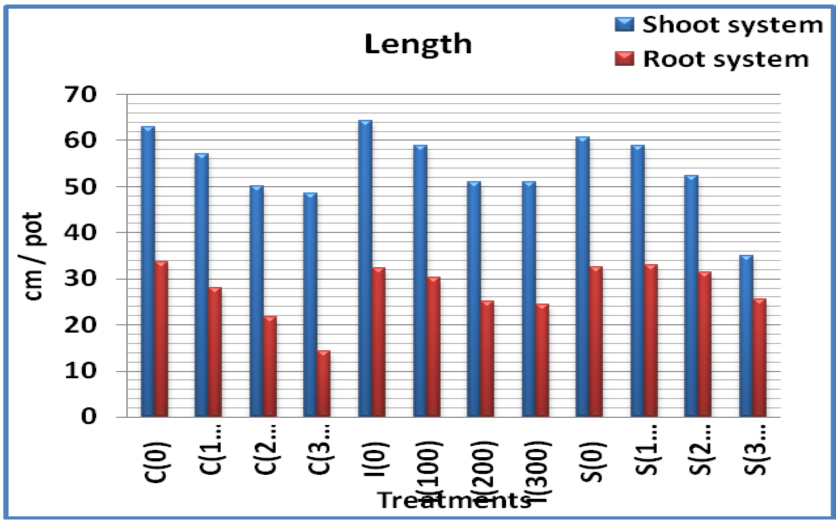
Statistical analysis of the data was conducted according to the analysis method used in a completely randomized design, and the means were isolated using the least significant difference test at the 5% significance level, Gomez, K.A. and Gomez, A.A. (1984)

RESULTS AND DISCUSSION:

Under pot experiment conditions, wheat (*T. aestivum* L.) was inoculated with *Azospirillum* sp. or Salicylic acid application at stress levels (0, 100, 200, and 300 m*M* NaCl). The obtained were presented in Fig. 2-3 show that *Azospirillum* sp. Inoculation or Salicylic acid applicationresulted significant increases in the growth parameters (length, fresh and dry weight, leaf-area) At all transaction levels compared to the standard transaction.Maximum stimulatory effect was obtained by *Azospirillum* sp. Inoculation at 100 m*M*NaCl and recorded the highest values of shoot and root height, leaf area recording 59.0, 30.3cm / pot and 87.5Cm^2 / plant, respectively. Also, Salicylic acid foliar application are increased in the (fresh and dryshootweight ; fresh and dryroot weight) by increasing treatments salinity, recorded 9.9, 1.9, 3.0 and 0.8 g / pot at 100 m*M* NaCl.

Results of this study demonstrated that wheat plant (*Triticum aestivum* L.) tolerated NaCl salinity up to the level 300 m*M*. Generally, reduction in the growth parameters of control un-inoculated plants was accompanied with increasing NaCl salinity,because salinity plant death. This inhibition in growth is ascribed to the metabolic disorder induced by NaCl salinity.

The *Azospirillum* sp. or Salicylic acid in the plants at all stress concentrations.When inoculated sorghum plants were exposed to water stress, the harmful effects on plant growth were reduced (Sarig *et al.*, 1992). Similarly, Dolatabadian *et al*., 2010)Spraying plant leaves with salicylic acid improved the growth of corn plants under water stress. Spraying plant leaves with salicylic acid also led to non-significant increases in seedling growth traits of sunflower plants grown under five levels of drought stress conditions reported by Sgherri and Navari-Izzo 1995.



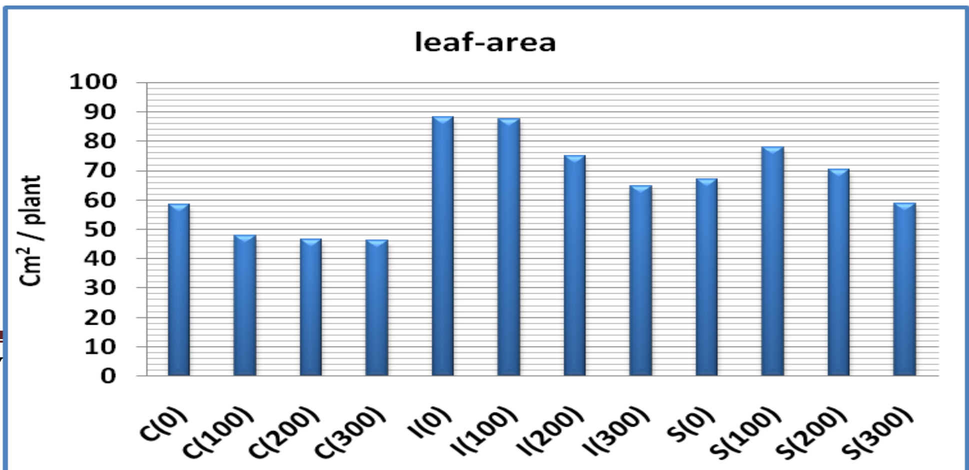
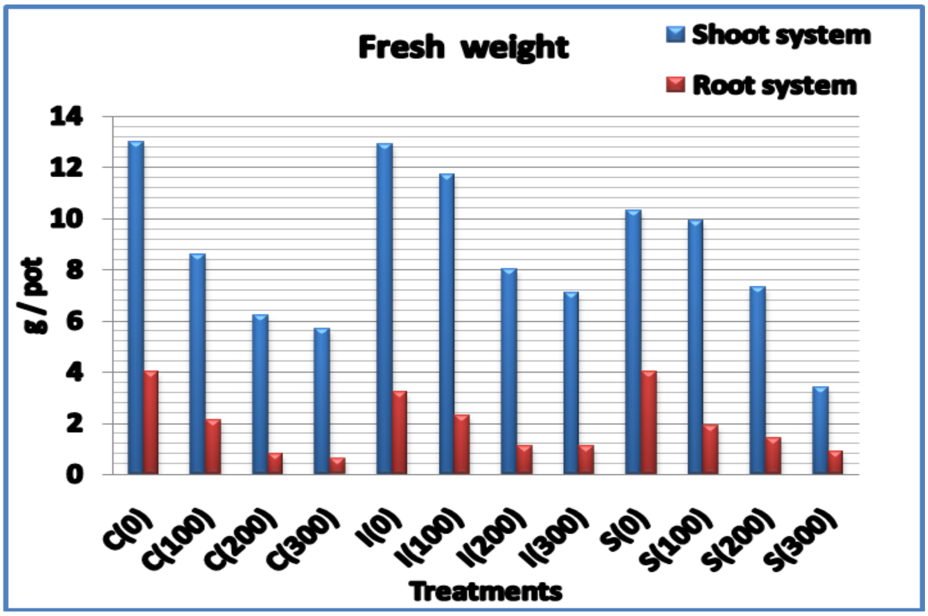


Fig. 2: Effect of applying treatments on some wheat plant growth characteristics



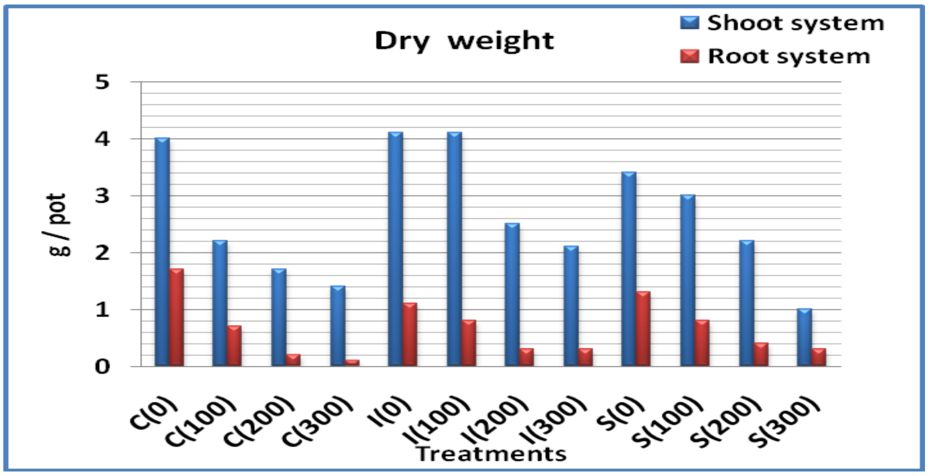


Fig. 3: Effect of applying treatments on some wheat plant growth characteristics

Chlorophyll *a*, chlorophyll *b* of *Triticum aestivum* L. grown at different NaCl levels gradually decreased with increasing salinity level in the soil (Fig. 4). This reduction was more prominent at the higher saline levels (300 mM NaCl).Application of the bio fertilizersand NaCl salinity the biosynthesis of pigments, but also induced a pronounced increase in their contents. *Azospirillum* sp. Inoculation increased pigments content (chl. a, b and Carotenoid) up to the level of 100 mM NaCl, and recording 2.46, 2.72 and 2.51mg/g compared with the control plant, respectively. In addition, Salicylic acid foliar application increased in pigments content and recorded 2.94, 2.80 and 2.58 mg/g at 100 m*M* NaCl.

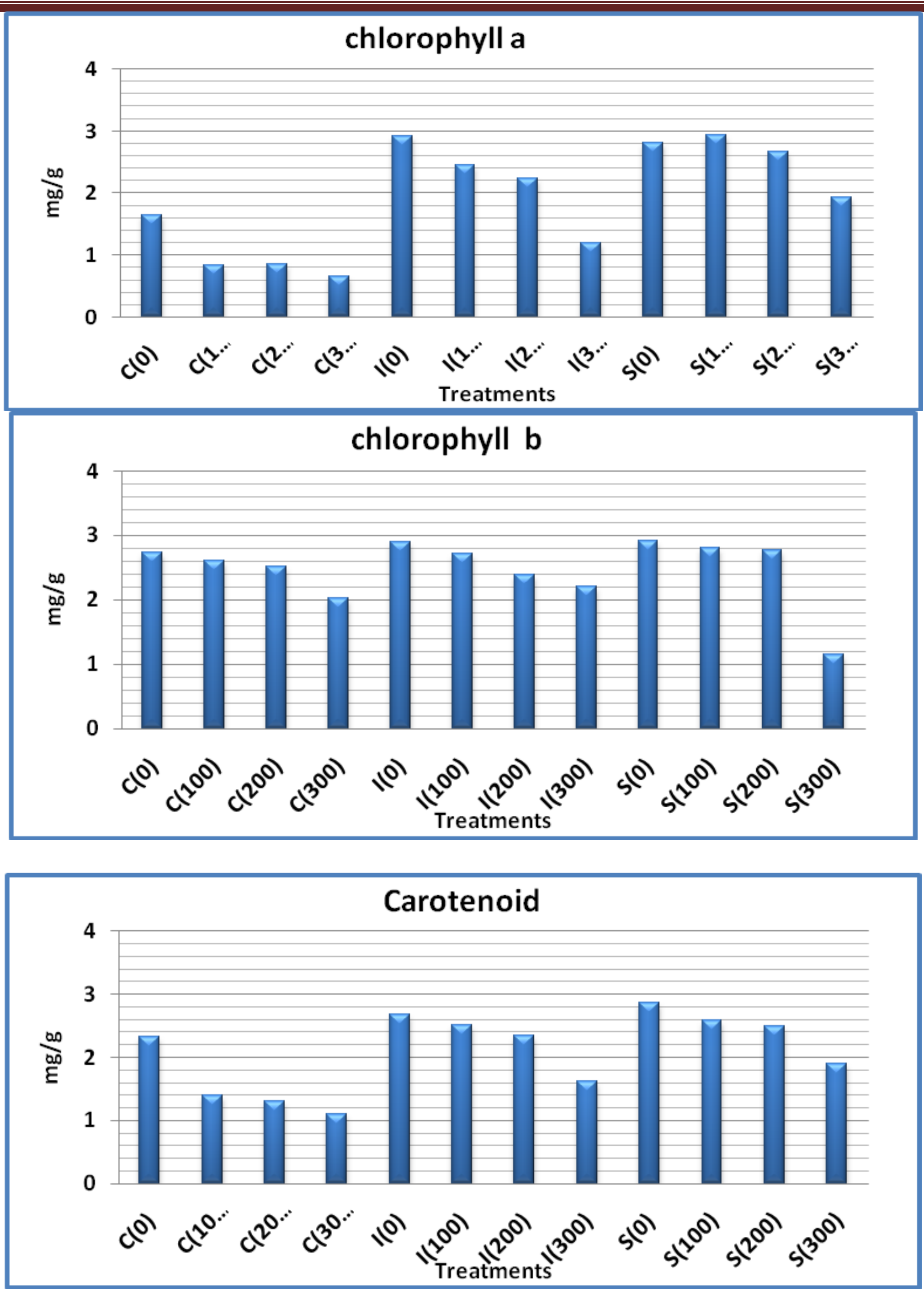


Fig. 4: Effect of applying treatments on some wheat plant growth characteristics

The results were given shown in figure 5 It indicates the percentage of carbohydrates in the plant as a whole It decreased as a result of increased salinity compared to untreated control plants. However, *Azospirillum* sp. inoculation or Salicylic acid application highly enhanced shoot and root soluble carbohedrates at 100 m*M* NaCl and recording 5.0, 4.2, 5.4 and 4.4 mg/g compared with the control plant, respectively.

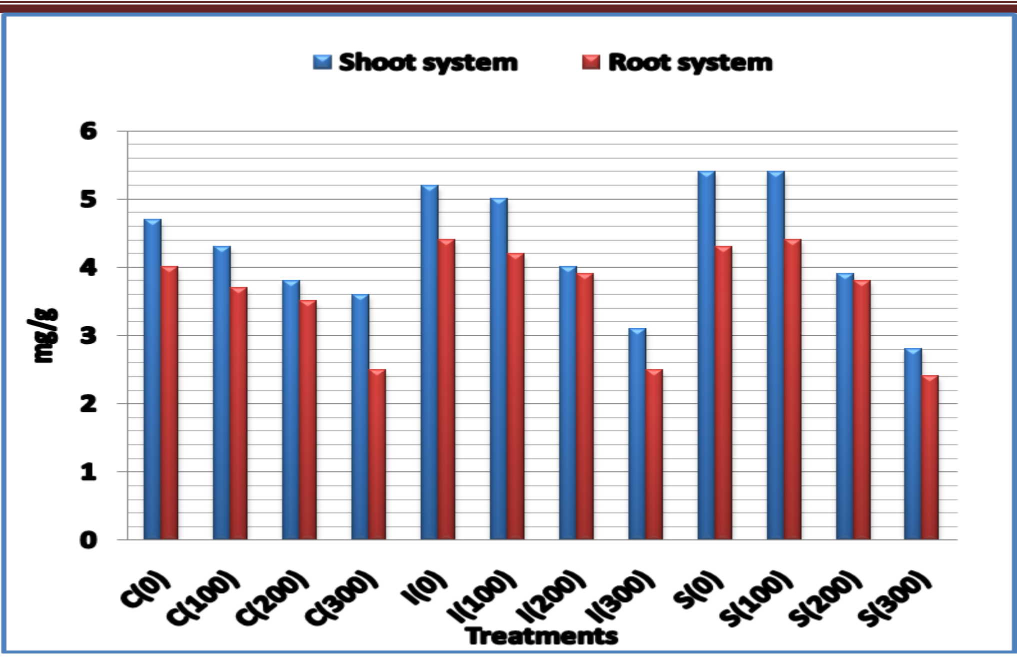
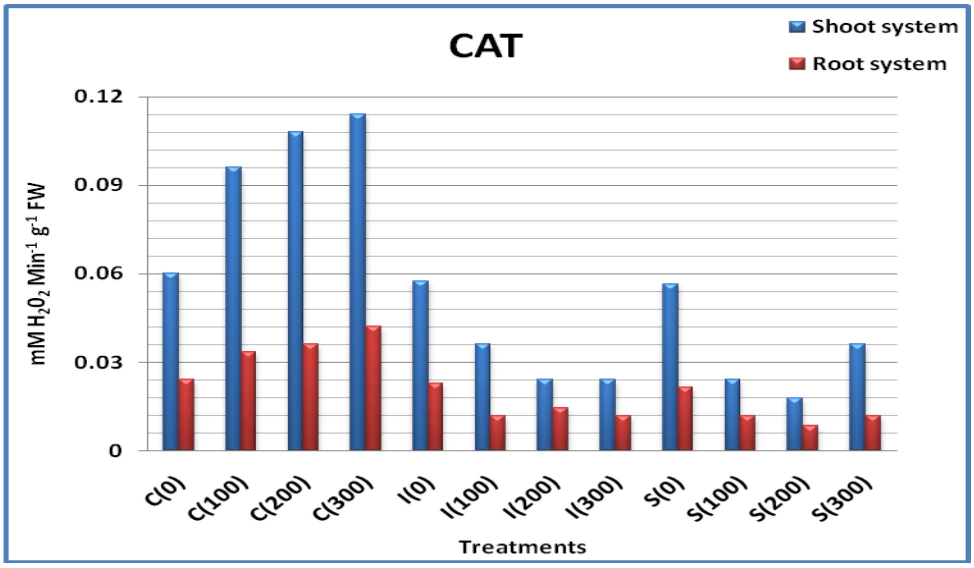


Fig. 5: Effect of applying treatments on some wheat plant growth characteristics

The present study showed that significant reduction in enzyme activity of un-inoculated plants exposed to salt stress by presence of addition H_2O_2. On contrast, the *Azospirillum* inoculated plants elevated the activity of CAT, POD when compared to the control. Salicylic acid caused decrease H_2O_2 levels are related to increased enzyme activities of shoot and root of *T. aestivum* L. Plant cells presumably regulate H_2O_2 levels by coordinating activities of H_2O_2 degrading enzymes such as CAT, POD (Mellouk *et al.* 2016). The POD activity was significantly increased under salt stress in *Pseudomonas* and *Azospirillum* inoculated Sunflower plant (Naz and Bano, 2013).



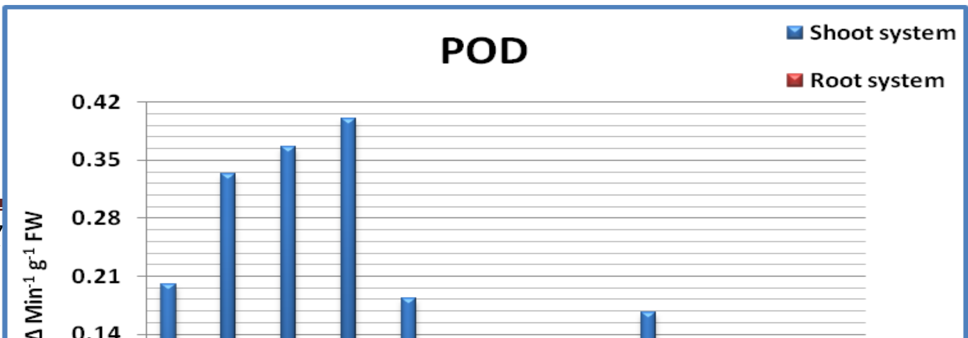


Fig. 6: Effect of applying treatments on some wheat plant growth characteristics

In present investigation hydrogen peroxide correlated increment was recorded in shoots and roots of wheat plants as compared with that of non-Stalinized control at all levels of NaCl (Fig.7) . Using H_2O_2 accumulation in plant tissues as an index for stress severity and its recovery was applied successfully in our laboratory (Mousa, 2014).

Mahesh *et al*., (2013) found in radish water stressed seedlings that there was a marked increase in H_2O_2 content reflecting lipid peroxidation and loss of membrane integrity. Hossain *et al*., (2013) reported that under Stalinized culture condition H_2O_2 content exhibited elevated levels in two rice genotypes with a higher magnitude in salt sensitive. Application *Azospirillum* sp. Inoculation or Salicylic acid on wheat plant resulted in decline in hydrogen peroxide accumulation and lipid peroxidation levels. The decrease in the levels of H_2O_2, as key indicators of stress indication demonstrates the efficient stress management by *Azospirillum* sp. Inoculation or Salicylic acid application. Ravkumar *et al.*, (2002) recommended the saline tolerant *Azospirillum* sp. for improving crop yield in coastal agricultural fields.

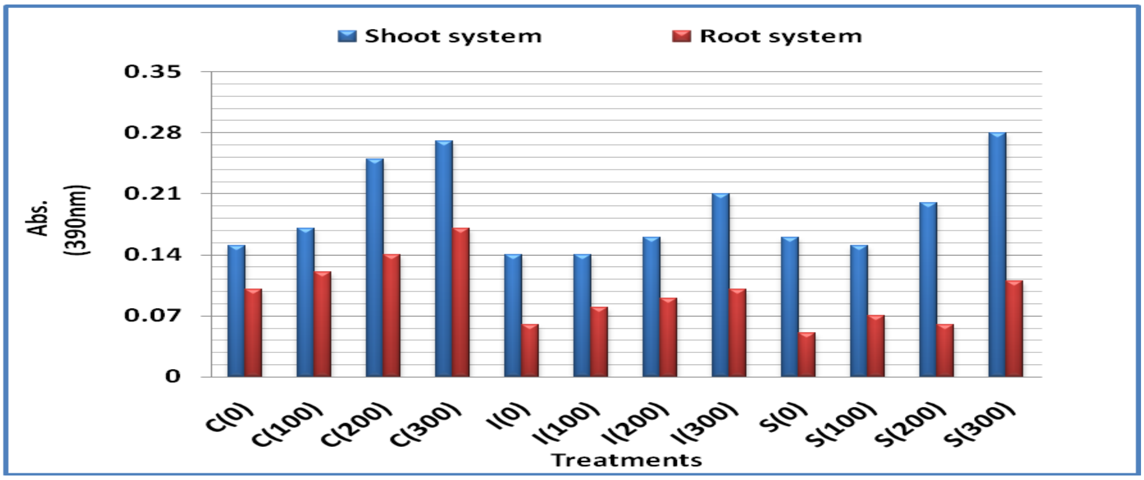


Fig. 7: Effect of applying treatments on some wheat plant growth characteristics

Results presented in Fig. 7 and representative in figure Fig. 8 Show that total shoot and root N-yield were Dropped by the NaCl as in the control non-saline treatment. However, *Azospirillum* sp. Inoculation resulted in the accumulation of N-Yield and fixed nitrogen amounting 30.6, 12.9, 6.6 and 2.4 mg N/pot in shoot and root at 200 m*M* sodium chloride, respectively. However, SA at 100 m*M* sodium chloride showed the highest % nitrogen derived atmosphere (%NdFa), which amounting 33.3 % and 20% in the shoot and root system. El-Komy *et al*. (2004) demonstrated that using the difference method for quantification of %NdFa in maize inoculated with *Azospirillum sp.* and grown under salt stress that the plants derived up to 28% and 17.6% of their shoot and root nitrogen from the atmosphere.

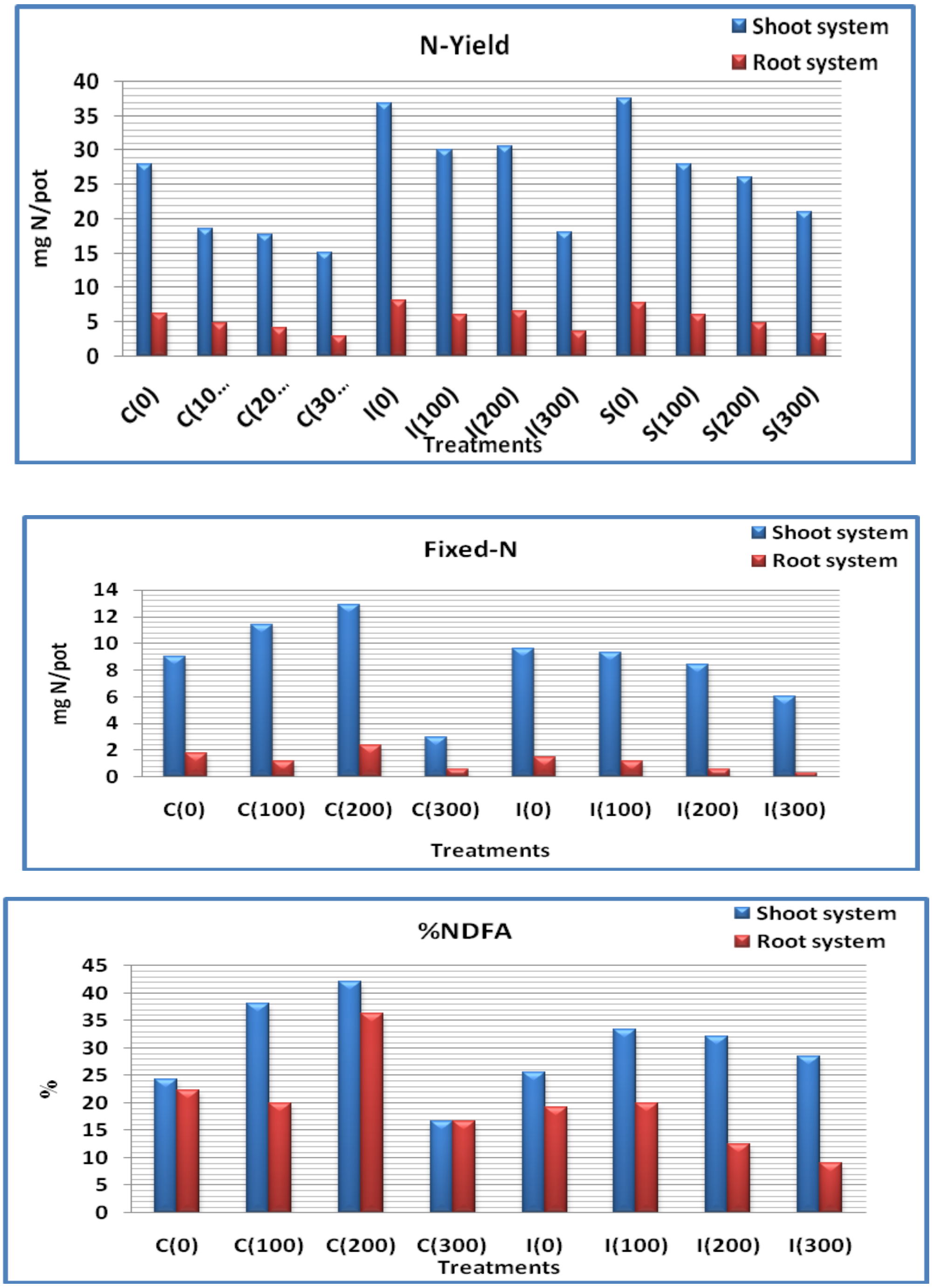
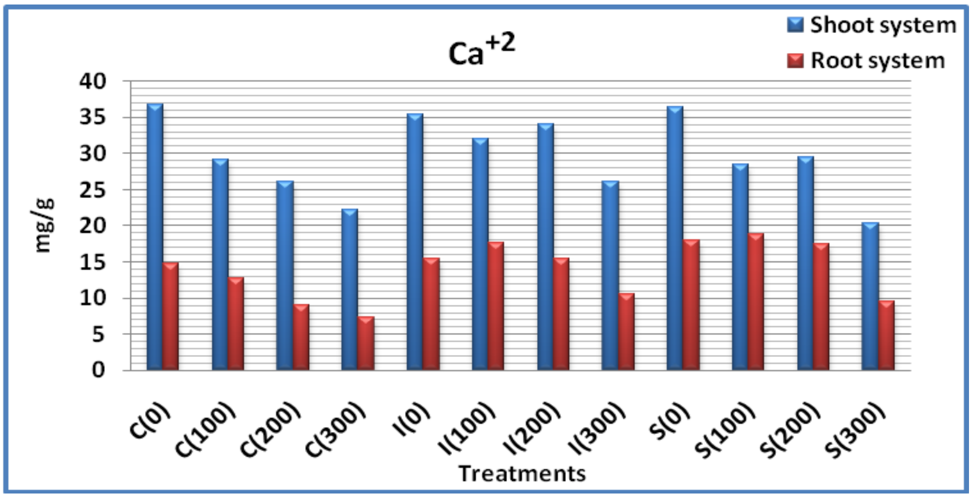
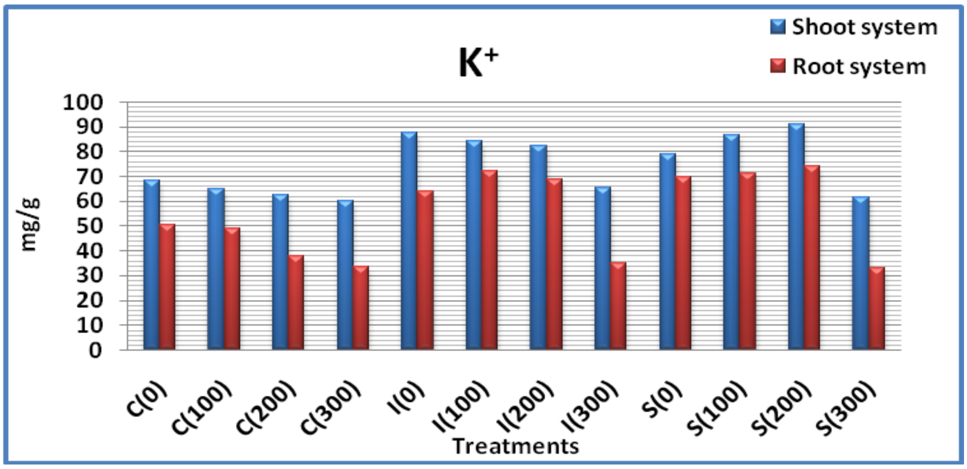


Fig. 8: Effect of applying treatments on some wheat plant growth characteristics

Results presented in Fig.8 and 9 indicated that inoculation of wheat with *Azospirillum* inoculation and salicylic acid application enhanced Ca^{+2} , K^{+} and phosphorus accumulation in shoot and root tissues under different levels of NaCl compared to control un-inoculated plants. Ca^{+2} accumulation increased significantly by *Azospirillum* inoculation and salicylic acid application as compared with the control plant up to the highest NaCl level (200 mM) and recorded 34.1 and 29.4 mg/g in shoot systems, respectively. Potassium accumulation enhanced with addition salicylic acid recorded 90.8 and 74.0 mg/gram of whole plant, 200 m*M* NaCl concentration, respectively. The change in the contents of minerals could be regard as a means of osmotic regulation in plant tissues (Hamdia and El-Komy, 1998; Abdel-Samad, 2005).





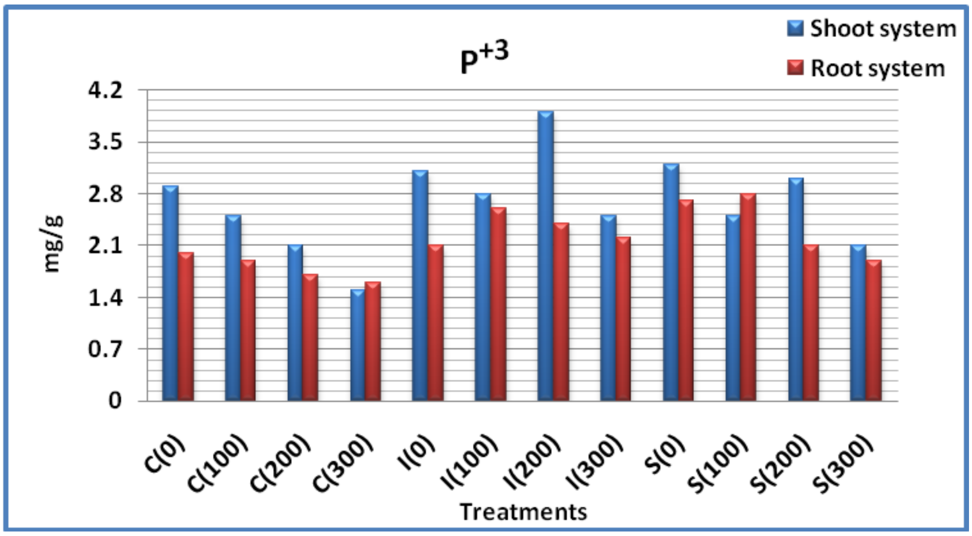


Fig. 9 : Effect of applying treatments on some wheat plant growth characteristics

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