International Letters of Natural Sciences *Online: 2014-08-05*
ISSN: 2300-9675, Vol. 22, pp 1-15
doi:10.56431/p-p7f51d

Effects of NaCl, $CaCl_2$ and their combination of salt on seed germination and seedling growth of *Lycopersicum esculentum* L.

S. Sivasankaramoorthy

Department of Botany (DDE), Annamalai University, Annamalai Nagar - 608 002, India

E-mail address: drsivasankarmoorthy@gmail.com

ABSTRACT

To compare the effect of NaCl, $CaCl_2$ and their combinations on germination and early seedling growth stages of *Lycopersicum esculentum L.*, were studied under pot experiments 2008. Results indicated that significant increases were recorded in percentage of germination, seedling fresh and dry weights, seedling length, water content, catalase activity and photosynthetic pigments (chlorophyll a, b and total chlorophylls as well as carotenoids) under the low level concentration (20 mM) of NaCl or CaCl and their combination (1:1). On other hand increasing salt concentration in nutrient solution caused significant decrease in all of these parameters. The great reduction occurred under high salinity level of NaCl (50 mM). Meanwhile, peroxidase activity increased significantly with increasing salinity levels from 20 mM to 50 mM of both applied salinity types. Besides, peroxidase activity under NaCl salinity showed a marked increase followed by NaCl + $CaCl_2$ (1:1) and $CaCl_2$ at 50 mM.

***Keywords*:** *Lycopersicum esculentum* L.; sodium chloride; calcium chloride; chlorophyll; catalase and peroxidase

1. INTRODUCTION

Lycopersicum esculentum L. is one of the most important stable crops for the world human nutrition. The arid and semi-arid conditions as well as less availability of fresh water have inflicted the saline condition in these provinces and are threatening the productivity of this crop, which is considered as moderately sensitive to salt stress (Lycoskoufis et al., 2005). In general, seed germination and early seedling growth are considered as the most sensitive stages to salinity stress (Ashraf and Foolad, 2005) in most of the crops than the growth of established. Seed germination and seedling growth of *Lycopersicum esculentum L.*, like other crops, were negatively affected by salinity stress (Khan et al., 2006 and Bassuony et al., 2008). Germination and emergence of *Lycopersicum esculentum L.*, seeds are also slow and non-uniform under normal as well as stress conditions (Demir and Okcu, 2004).Soil salinity, if not property managed, causing decrease in germination rate and germination percentage of *Lycopersicum esculentum L.*

The salt damage to the seed germination is attributed to various factors such as reduction in water availability, changes in mobilization of stored reserves and affecting

structural organization of proteins (Almansouri et al., 2001). The seeds require higher amount of water uptake during the germination under the salt stress due to the accumulation of the soluble solutes around the seeds, which increases the osmatic pressure. This causes excessive uptake of the ions which results in toxicity in the plant. Moreover, water potential gradient (reduced water availability) between the external environment and the seeds also inhibits the primary root emergence (Delachiave and DePinho, 2003). The most important process that is affected in plants, growing under saline conditions, is photosynthesis.

Reduced photosynthesis under salinity is not only attributed to stomata closure leading to a reduction of intercellular CO_2 concentration, but also to non-stomata factors. There is strong evidence that salt affects photosynthetic enzymes, chlorophylls and carotenoids (Stepien and Klobus, 2006). In addition, salinity causing disturbance of membrane integrity (Hasegawa et al., 2000) activities of enzymes and damaged photosynthetic components (Winston, 1990). Moreover, plants have the ability to scavenge/detoxify reactive oxygen species by producing different types of antioxidants (enzymatic and non-enzymatic). Enzymatic antioxidants such as catalase, peroxidase, (Prochazkova and Wilhlmova, 2007 and Ashraf, 2009). Therefore, the present study was planned to determine the effects of NaCl, $CaCl_2$ salinity and their combinations on germination and growth of *Lycopersicum esculentum L*.

2. MATERIALS AND METHODS

The experiment was carried out in the laboratory of the Agricultural Botany Dept., Fac. of Agriculture, Mansoura Univ. during the growing season 2008, to study the effect of NaCl of $CaCl_2$ and their combination (1:1 w/w) on germination and early seedling growth stages of sweet pepper (*Capsicum annuum* L, cv Orlando), a hybrid 'California Wonder'. The seeds used in this investigation were secured from the Gohara Co. Cairo, Egypt. Salinity stress was induced by Sodium Chloride (NaCl), Calcium Chloride ($CaCl_2$) and their recombination, NaCl: $CaCl_2$ (1:1 w/w) from EL- Gomhoria Co., Egypt and were used at the concentrations of 2000 and 4000 ppm each.

A homogenous lot of sweet pepper seeds were placed in 100 ml beakers and 20 ml of 1 % sodium hypochlorite was added for sterilization. These were left in the solution for 5 min followed by washing under running tap water and ionized water twice, then soaked (24 hours) in distilled water. After soaking, the sterilized seeds were divided into 7 sets (7 salinity levels), then placed in glass Petri dishes (11 cm) (25 seeds/dish) with a double layer. Of Whatman No. 1 filter paper. The first set was moistened with 10 ml nutrient Cooper solution. (Cooper, 1979) E.C., (2.0 dSm^{-1}) served as control. The six remainder sets were salinized with 10 ml nutrient solution adding salts. Measuring the electrical conductivity by digital conductivity meter Lutron CD-4301.

The dishes were left in an incubator in the bark for seed germination at 25 $\pm$2 °C and 90 % relative humidity, then the dishes were covered with aluminum foils for darkens. In order to avoid water losses, 5 ml of the nutrient solution were added to Petri dishes, every 5 days. Thiram was added to the solution at a concentration of 2 % (w/v) to control the fungi infection. The experiment was repeated two times and arranged in a completely randomized block design with three replicates.

a. Seeding fresh and dry weight (g/5 seedling).

b. Seedling length (cm).

c. The seedling water content (WC) expressed as (mg g^{-1}) was calculated from FW and DW values (Song and Fujiyama, 1998) using the following equation: WC = [(FW-DW)/FW] +1000. Where WC is the water content (g g^{-1}); FW and DW are the fresh weight (g $seedling^{-1}$) and dry weight of the (g $seedling^{-1}$) of the seedlings plant, respectively.

d. Enzymatic activity: the enzyme extraction was done as recommended by Maxwell and Bateman (1967). One gram of fresh sample (cotyledons) was ground with 10 ml 0.1 M Na-phosphate buffer at pH 7.1 in a lab mortar. The homogenate was transferred to centrifuge tubes and was centrifuged at 4C for 15 min at 15000 rpm in laboratory refrigerated centrifuges model SIGMA 4K15. The supernatant was made up to a known volume with the same buffer and used for enzyme assay.

d. 1. Peroxidase activity (EC1.11.1.7): the activity of Peroxidase activity was determined with a (Spekoll 11) spectrophotometer. The activity of Peroxidase activity was determined according to the method described by Allam and Hollis (1972). This method depends on measuring the oxidation of pyrogallol to pyrogallin in the presence of hydrogen peroxide (H_2O_2) at 425 nm. The sample cuvette contained 500 µl of 0.1 M potassium phosphate buffer (pH 7.0) + 300 µl of 0.05 M pyrogallol (6.3 g/L) + 100 µl of 1.0 % H_2O_2 + 100 µl enzyme extract. Readings were recorded every 30 seconds for 5 minutes at 27 $\pm$2 °C. The activity was expressed as ΔA_{410} g^{-1} min^{-1}.

d. 2. Catalase activity (EC1.11.1.6): the enzymatic activity of CAT was measure according to Aebi (1984). About 3 ml reaction mixture containing 1.5 ml of 100 mM potassium phosphate buffer (pH 7.0), 0.5 ml of 75 mM H_2O_2, 0.05 ml enzymes extraction and distilled water to make up the volume to 3 ml. The reaction started by adding H_2O_2 and a decrease in absorbance was recorded at 240 nm for 1 min. Enzymes activity was computed by calculating the amount of H_2O_2 decomposed. Each enzyme activity was expressed as enzyme unit per gram fresh weight of leaf.

e. Photosynthetic pigments (mg/g FW): fresh leaf samples (0.05 g) were extracted by methanol for 24 hours at laboratory temperature after adding a trace from sodium carbonate (Robinson et al., 1983), then chlorophyll a, b and carotenoids were determined spectrophotometrically (Spekol II) (at wave lengths 452, 650, 665 nm). The quantities of total chlorophylls, chlorophyll a, carotenoids concentration (mg/g) in leaves were determined by the equations proposed by Mackiny (1941).

$$\text{Total Chlorophyll} = (25.5^{*} E_{650} + 4^{*} E_{665})^{/5}$$

$$\text{Chlorophyll a} = (16.5^{*} E_{665} - 8.3^{*} E_{650})^{/5}$$

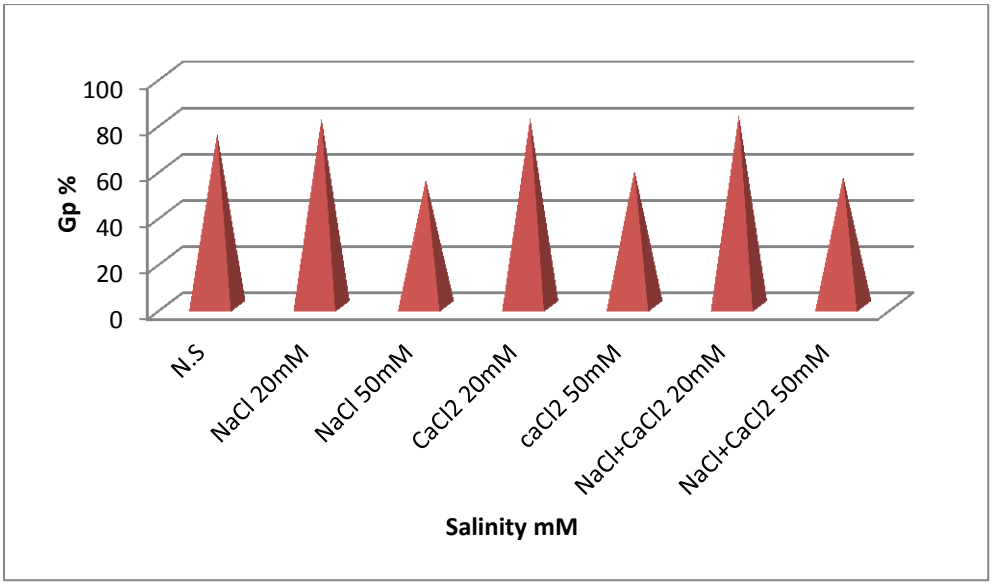
$$\text{Chlorophyll b} = (33.8^{*} E_{650} - 12.5^{*} E_{665})^{/5}$$

$$\text{Carotenoids} = (4.2^{*} E_{452.5}) - (0.0264^{*}\text{Chl. a}) - (0.496^{*}\text{Chl. b})^{/5}$$

3. RESULTS AND DISCUSSION

1. Germination percentage: Generally, increasing salinity causes a decrease in maize germination; this might be due to the toxic effects of Na^+ and in the process of germination (Khajeh-Hosseini et al., 2003). It alters the imbibitions of water by seeds due to lower osmotic potential of germination media, causes toxicity which changes the activity of enzymes of nucleic acid metabolism changes protein metabolism, interrupts hormonal balance, and reduces the utilization of seed reserve food (Gomes-Filho et al., 2002). Primed seeds of maize might have better competency for water absorption from the growing media that enabled metabolic activities in seeds during germination process of a start much earlier than radical and plumule appearance (Elouaer and Hannachi., 2012).

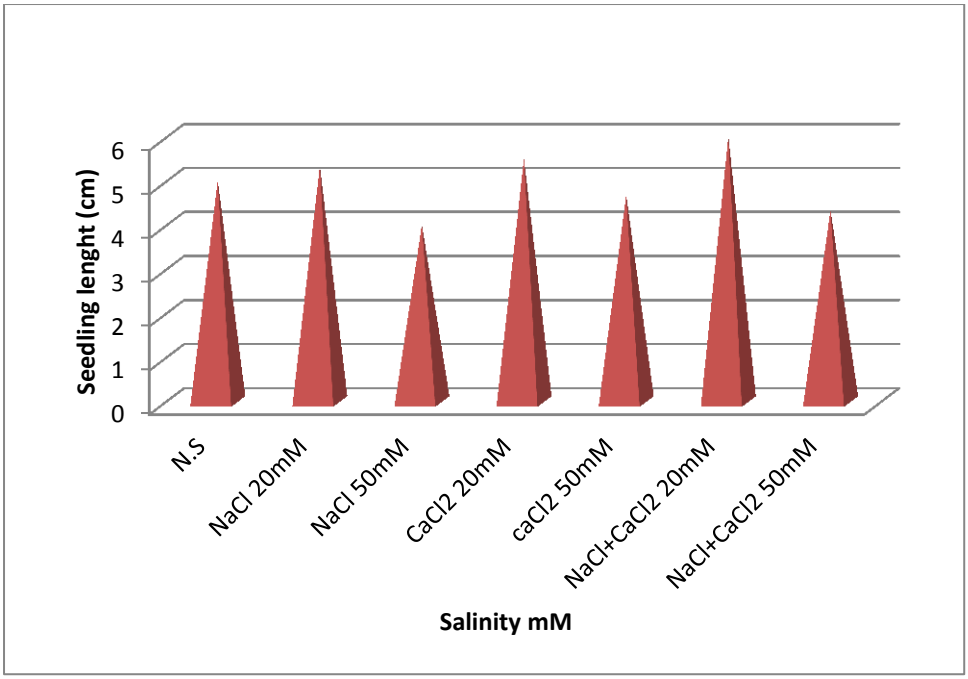
Figure 1. Germination percentage of *Lycopersicum esculentum* under normal or saline condition (NaCl, $CaCl_2$ and their combination) after 15 days from sowing.



2. Seedling growth: The data illustrated in Figures (2.5) and shown in plate (1) indicate that fresh and dry weights, seedling length as well as water content after 14 days were increased significantly under low levels 2000 ppm of all salinity types. On the other hand, the increasing salt concentration in nutrient cooper solution caused significant decrease in fresh and dry weights as well as seedling length but seedling water content showed no-significant effect. The great reduction occurred under high salinity level of NaCl. In the present investigation, Reduced seedling length under saline condition may be due to accumulation of toxic ions, that facilitates the intake ions in sufficient amounts to be toxic for the embryonic activities due to the influence of the cations more anions, the entry of ions to the seeds that might have been toxic to the embryo or the developing seedlings (Almodares et al., 2001) and / or inhibition of the uptake of several essential nutrients causing nutritional or ionic imbalance (Taamalli et al., 2004) and /or disturbance in metabolic metabolism leading to an increase in phenolic compounds (Ayaz, et al., 2000) and / or which led to decreasing both cell division and cell elongation.

Salinity induced osmotic cell enlargement depending on soluble accumulation and its effect on cell size and number of cells per unit area (Greenway, 1963). The effects of salinity on seedling shoot and root length may be due to the negative effects of salinity on meristematic cell division and elongation as well as root penetration (Hatung, 2004) and due to reduced cell division or cell enlargement caused by salinity stress (Hawker and Walker, 1978).

Figure 2. Seedling length (cm) of *Lycopersicum esculentum* under normal or saline condition (NaCl, $CaCl_2$ and their combination) after 15 days from sowing.



The reduced seedling fresh weight and dry weight under salt stress conditions could be attributed to the water potential and osmotic adjustments as result of increased ionic concentration in their shell or bound water (Schwarz, 1985) and /or osmotic adjustment needed to keep root water potential lower than that of the external medium, energy must be expended to create such osmotic adjustment and this may lead to seedling growth reduction (Yeo, 1983) and /or might be attributed to the osmotic effect resulting from salt stress which causes disturbances in water balance and inhibits apical growth and internal imbalance (Younis et al., 2003 and Hegazy, 2009).

And /or inhibits cytokines biosynthesis and hormonal unbalances, reducing water content and some plant nutrients uptake as well as biosynthesis and hormonal unbalance, reducing water content and some plant nutrients uptake as well as biosynthesis of α-tocopherol, ascorbic acid and net photosynthetic rate accompanied with high respiration rate were also reported under stress conditions (Tripathi et al., 2007) and /or may be due to toxic effects unbalanced nutrient uptake by the seedlings (Hajibagheri et al., 1989) and /or decreases in water content have been communicated for many seedlings growing under salinity (Meloni et al., 2008).

Figure 3. Fresh weight (g/5 seedling) of *Lycopersicum esculentum* under normal or saline condition (NaCl, $CaCl_2$ and their combination) after 15 days from sowing.

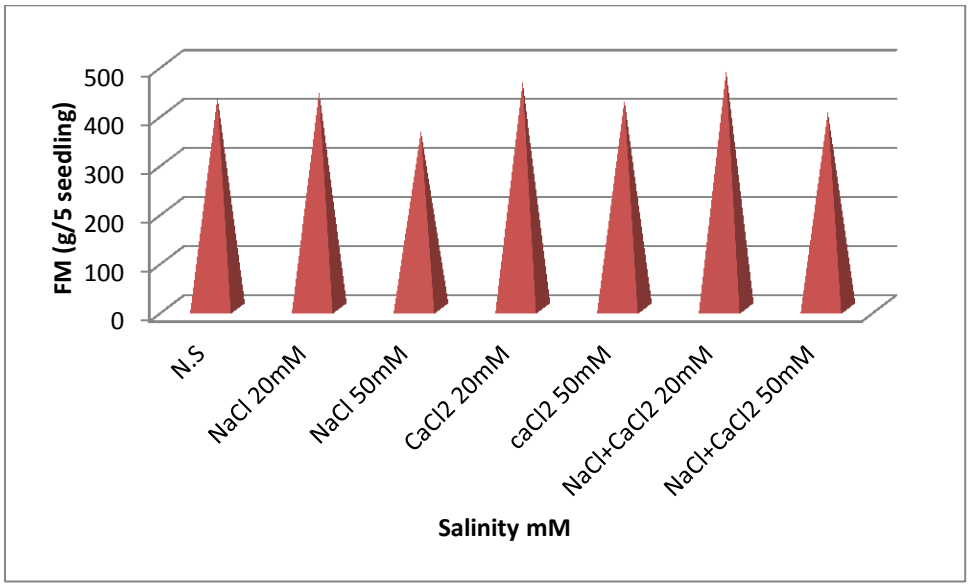
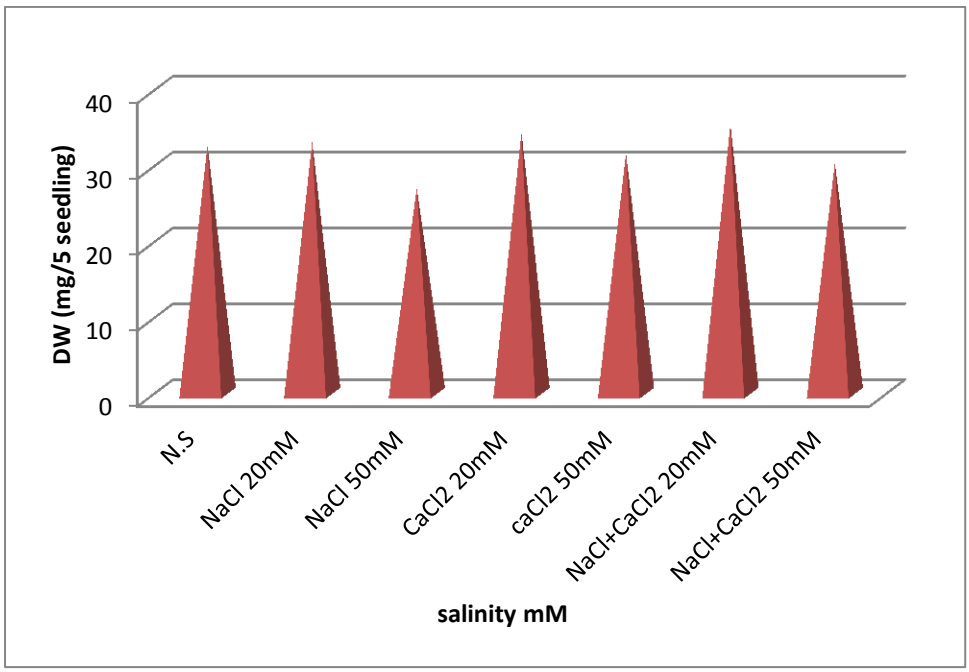


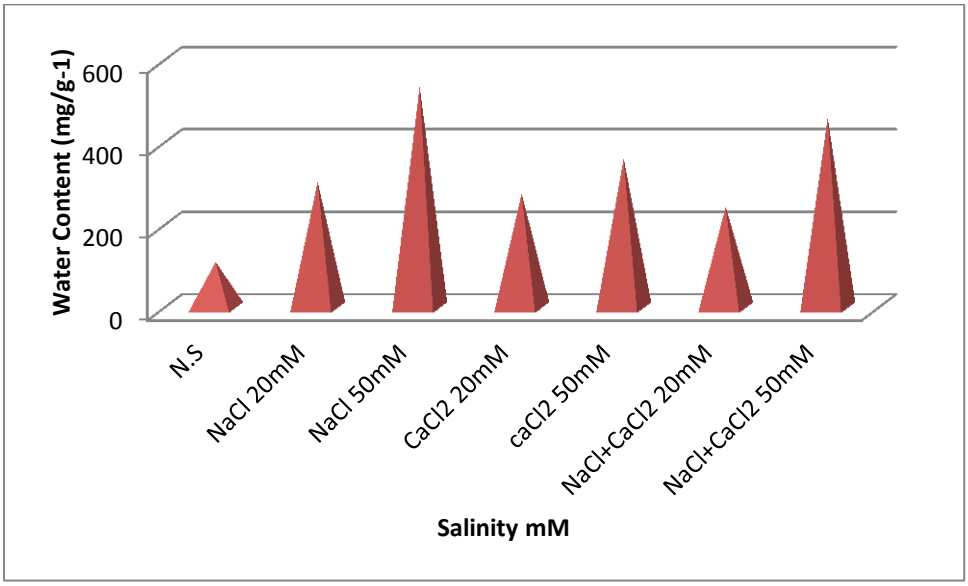
Figure 4. Dry weight (g/5 seedling) of *Lycopersicum esculentum* under normal or saline condition (NaCl, $CaCl_2$ and their combination) after 15 days from sowing.



The water content, although a convenient and widely used methods of assessing plant water status, is nit useful indicator of turgor in salt-treated plants undergoing osmotic adjustment. In most plants, especially halophytes, the solute content of cells at high salinity is higher than in non-saline condition, due largely to accumulation of ions (e.g. Na^+ and Cl^-)

and organic solutes. Therefore, during the rehydration to establish water content, the higher solute content in salt-treated than in untreated cotyledons causes a greater water uptake in the former than the latter. Thus, this fact results in an apparently low RWC under salinity (Munnus et al., 2006).

Figure 5. Seedling water content (mg/g^{-1}) of *Lycopersicum esculentum* under normal or saline condition (NaCl, $CaCl_2$ and their combination) after 15 days from sowing.



3. Peroxidase and Catalase activity: The data illustrated in (Figure 6) indicate that all applied salinity types increased significantly peroxidase activity of *Lycopersicum esculentum* seedling and high level of salinity was more effective in this respect. Moreover, peroxidase activity under NaCl stress showed a marked increase followed by NaCl + $CaCl_2$ (1:1) and $CaCl_2$.

Concerning the catalase activity (Figure 7) under low level of all applied salinity types (20 mM) increased significantly catalase activity and NaCl + $CaCl_2$ (1:1) proved to be more effective in this respect., followed by $CaCl_2$ and NaCl. On the other hand, increasing salinity levels to 50 mM decreased significantly catalase activity and the great reduction occurred under NaCl stress. Salt stress produced ROS is a common phenomenon which can interact with a number of destructive processes causing cellular damage (Ashraf, 2009), cell signaling, gene regulation, senescence, programmed cell death, pathogen defense, and others (Gechev et al., 2006).

Present results, the antioxidant enzymes peroxidase activity and catalase activity were increased under NaCl salinity (Figure 6) and further enhanced due to $CaCl_2$ treatment. These results are in agreement with those reported by (Jaleel et al., 2007). The plants defend against these reactive oxygen species by induction of activities of certain antioxidative enzymes such as catalase, peroxidase (Mittova et al., 2003). Catalase is specific to a great extent for H_2O_2, and removed excess H_2O_2 before it can leak out into other parts of the cell (Ali and Alqurainy, 2000). The high concentration of H_2O_2, while low concentration of H_2O_2, was mainly scavenged by peroxidase activity during the period of oxidation of relative substances.

Figure 6. Peroxidase activity (unit/g FW) of seedling *Lycopersicum esculentum* as affected by salinity (NaCl, $CaCl_2$ and their combination) after 15 days from sowing.

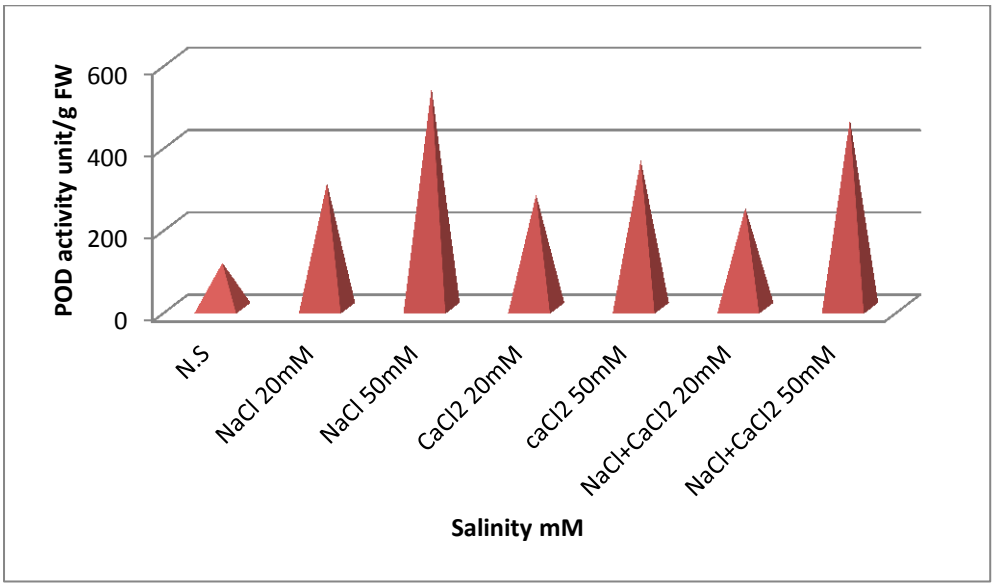
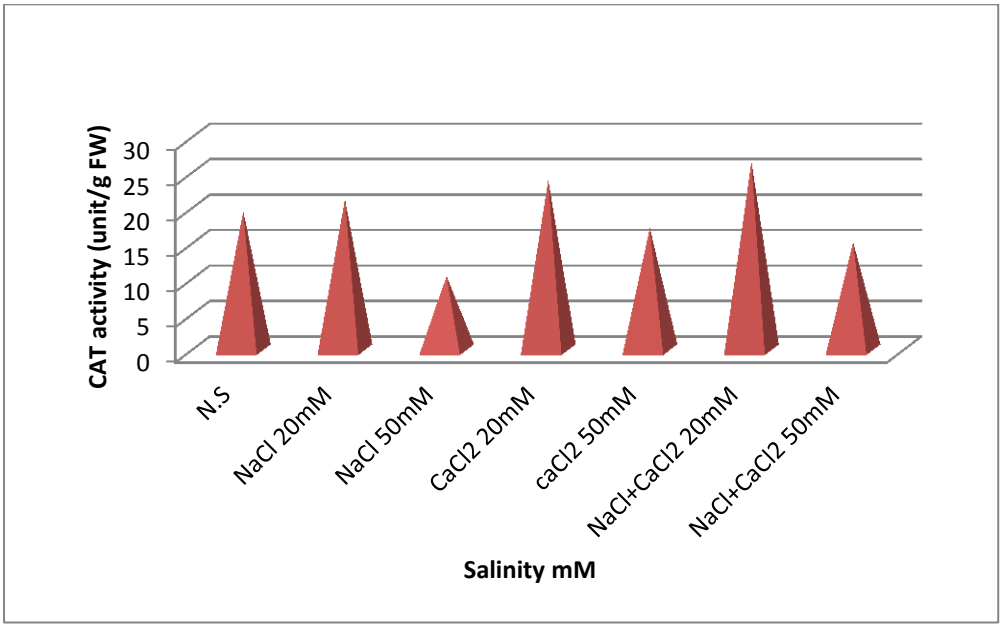


Figure 7. Catalase activity (unit/g FW) of seedling *Lycopersicum esculentum* as affected by salinity (NaCl, $CaCl_2$ and their combination) after 15 days from sowing.

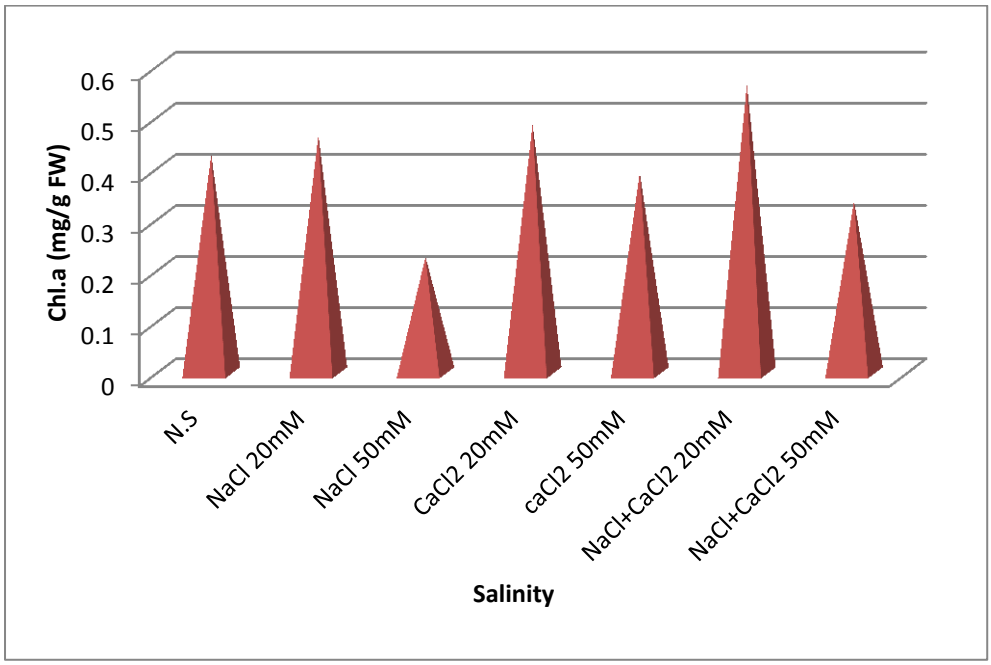


When peroxidase activity and catalase activity were consistent and in harmony with one another, free radicals from ROS in plants could be kept at a low level which exerted the plant growth and metabolize naturally (Jiang, 1999). In addition, Turhan et al., (2006) proposed that the peroxidase activity was increased coordinately in response to salt. In addition, Li (2009) revealed that, on tomato seedling the catalase activity increased under (100 mM NaCl), but the catalase activity decreased under 200-300 mM NaCl. Moreover,

Wang, et al., (2009) on alfalfa, found that salinity stress increased catalase activity. On the other hand, Noreen and Ashraf (2009) revealed the salt stress enhanced the activities of peroxidase activity while, decreased the catalase activity in pea. Also, Hassanein et al., (2009) found that, activity level of peroxidase activity enzyme progressively increased with increasing salinity levels, while the behavior of catalase activity showed an opposite response. In addition, Gadalla (2009) found that NaCl reduced the activity of catalase activity and peroxidase activity.

4. Photosynthetic pigments concentrations: The data illustrated in Figures (9-12) clearly show that low salinity level (20 mM) of all applied salinity types {NaCl, $CaCl_2$ and their combinations 1:1 (w: w)} caused a high significant increase in the photosynthetic pigments concentrations (chlorophyll a, b and total chlorophylls as well as carotenoids). In addition, NaCl+$CaCl_2$ (1:1) caused a greater increase in photosynthetic pigments concentrations followed by $CaCl_2$ and NaCl. Salinity leads to an increase in free radicals in chloroplast and this causes destruction of chlorophyll molecules by then resulting in reduced photosynthesis and growth (Lichtenthlaler et al., 2005). In the present study, salt stress reduced the total chlorophyll content of cucumber seedlings by 50 % at both the NaCl levels and this could be associated with seedling growth inhibition observed under salinity stress. As compared to non-primed seedlings, priming of seeds with $CaCl_2$ doubled the chlorophyll content under 100 mM NaCl whereas at 150 mM NaCl there was no significant increase. According to Montesano and lersel (2007), calcium prevents the toxic effects of NaCl on photosynthesis and this appeared to be the reason for priming induced improved chlorophyll content of seedlings of Cucumber sativa observed in our study, which is in agreement with Afzal et al., (2012). Yeo et al., (1991).

Figure 8. Chlorophyll a concentration (mg/g FW) of seedling *Lycopersicum esculentum* as affected by salinity (NaCl, $CaCl_2$ and their combination) after 15 days from sowing.



On the other hand, photosynthetic pigments concentrations decreased gradually with increasing salinity levels from 20 to 50 mM. The great reduction in photosynthetic pigments occurred under NaCl at high salinity levels (50 mM). While, $CaCl_2$ at 50 mM increased significantly the total carotenoids as compared with other salinity types. These results are in agreement with those recorded by Parida et al., (2004). Who reported that photosynthetic rate increased at low salinity level and decreased at the higher ones.

Figure 9. Chlorophyll b concentration (mg/g FW) of seedling *Lycopersicum esculentum* as affected by salinity (NaCl, $CaCl_2$ and their combination) after 15 days from sowing.

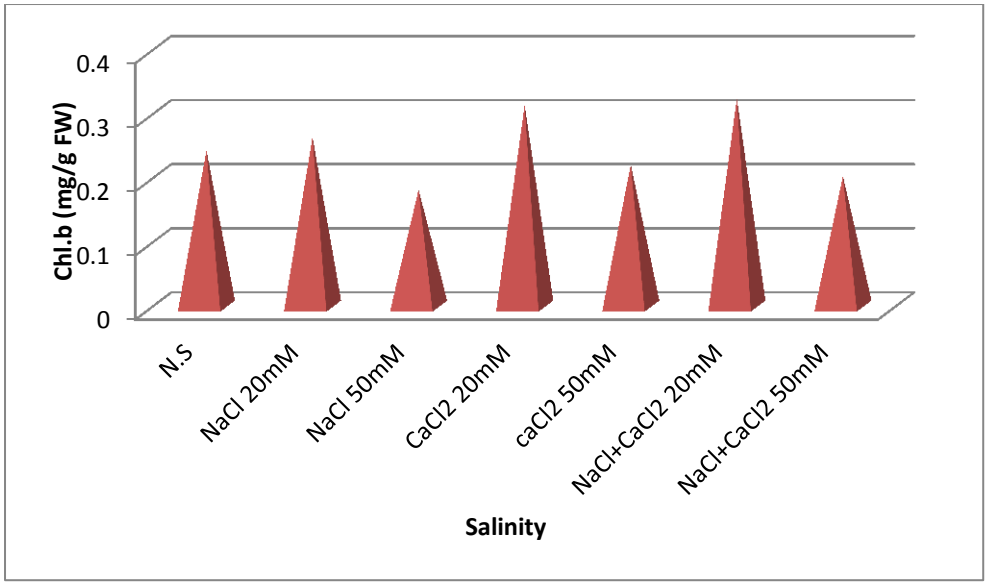


Figure 10. Total chlorophyll concentration (mg/g FW) of seedling *Lycopersicum esculentum* as affected by salinity (NaCl, $CaCl_2$ and their combination) after 15 days from sowing.

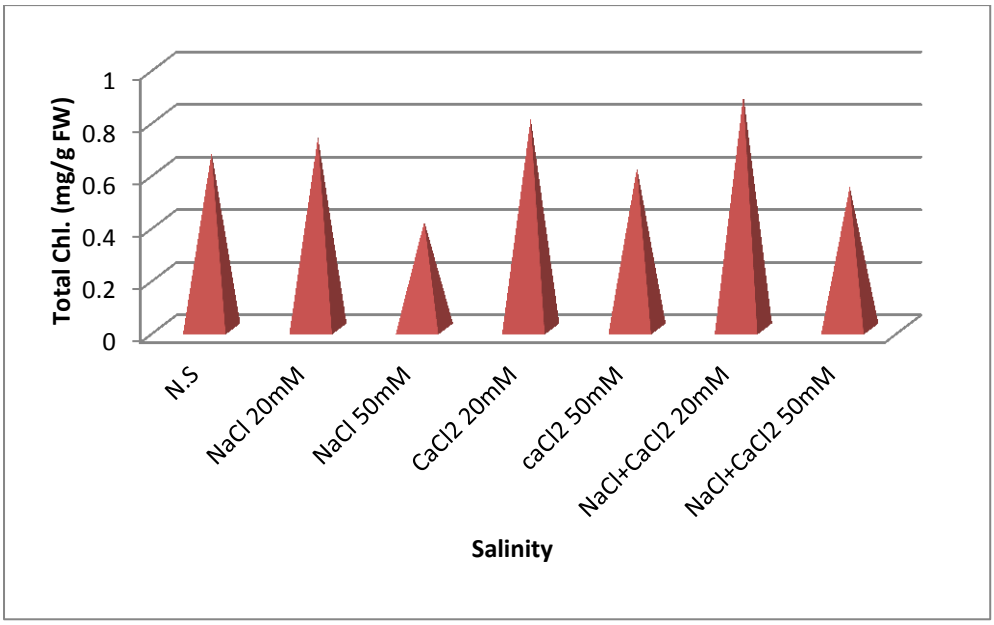
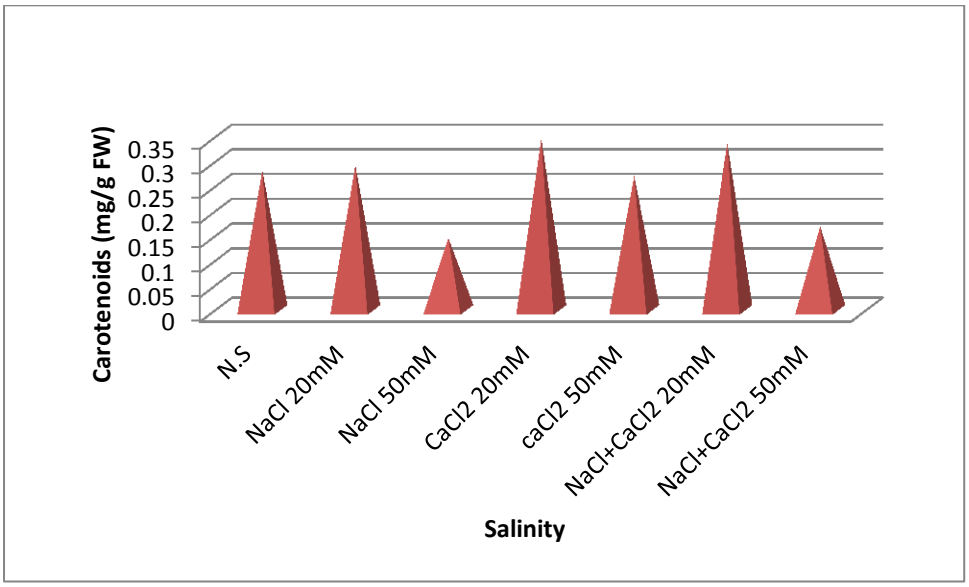


Figure 11. Carotenoids concentration (mg/g FW) of seedling *Lycopersicum esculentum* as affected by salinity (NaCl, $CaCl_2$ and their combination) after 15 days from sowing.



The reduction in photosynthetic pigments concentration under high salinity levels may be due to inhibitory effect of chloride on the activity of Fe containing enzymes, cytochrome oxidase which may decrease the rate of chlorophyll, biosynthesis and their accumulation (Helay et al., 1984) and /or enhancing the activity of chlorophyll degrading enzyme chlorophyllase (Mishra and Sharma, 1994) and/or oxidation of chlorophyll and decreased its concentration (Pell and Dann, 1991) and/or the toxic action of NaCl on the biosynthesis of pigments, increasing their degradation and /or maintaining damage of the chloroplast thylakoid (Hashem, 2000).

4. CONCLUSION

It could be concluded that low salinity level (20 mM NaCl) had a no effect to the *Lycopersicum esculentum* plants, on the other hand 50 mM NaCl significantly reduce the growth parameters. Application of $CaCl_2$ to NaCl stressed *Lycopersicum esculentum* significantly alleviated the effect of salinity stress.

References

[1] Lycoskoufis, I.H., Savas, D. and Mavrogianopouls, G. 2005. Growth gas exchange and nutrient status in pepper (*Capsium annuum L.)* grown in recirculating nutrient solution as affected by salinity imposed to half of the root system. *Scientia Hort.,* 106: 147-161.

[2] Ashraf, M. and Foolad. M.R. 2005. Per-sowing seed treatment a shotgun approach to improve germination, plant growth, and crop yield under saline and non- saline conditions. *Advances in Agronomy*, 88: 223-371.

[3] Khan, A., M.S.A. Ahma, H.U. Athar and M. Ashraf, 2006. Interactive effect of foliarly applied ascorbic acid and salt stress on wheat (*Triticum aestivum* L.) at the seedling stage. *Pak. J. Bot*., 38: 1407-1414.

[4] Bassuony, F.M. Hassanein, R.A. Baraka, D.M. and Khalil, R.R. 2008. Physiological effects of nicotinamide and ascorbic acid on *Zea mays* plant grown under salinity stress ii-changes in nitrogen constituents, protein profiles, protease enzyme and certain inorganic cations. *Aust. J. basic and App. Sci.,* 2(3): 350-359.

[5] Almansouri, M. Kinet, M. and Lutts, S. 2001. Effect of salt and osmotic stresses on germination in durum wheat (*Triticum durum Dest). Plant and Soil*, 231: 243-254.

[6] Delachiave, M.E.A. and De Pinho, S.Z. 2003. Scarification, temperature and light in germination of *Senna occidentalis* seed (Caesalpinaceae). *Seed Sci. and Techology*, 31(2): 225-230.

[7] Stepien, P. and G. Klobus, 2006**.** Water relations and photosynthesis in Cucumis sativus L. leaves under salt stress. *Biol. Plant*, 50: 610-616.

[8] Hasegawa, P.M., Bressan, R.A., Zhu, J.K, and Bohnert, H.J. 2000. Plant cellular and molecular responses to high salinity. *Annu. Rev. Plant Physiol. Plant Mol. Biol.,* 51: 463-499.

[9] Winston, G.W. 1990. Physio-chemical basis for free radical formation in cells: production and defenses, pp. 57-86

[10] Prochazkova, D. and Wilhelmova, N. 2007. Leaf senescence and activities of the antioxidant enzymes. *Boil. Plant*, 51: 401-406.

[11] Ashraf, M. 2009. Biotechnological approach of improving plant salt tolerance using antioxidants as markers. *Biotechnology Advances*, 27: 84-93.

[12] Cooper, A., 1979. The ABC of NFT. Growers Books, London, P.59.

[13] Song, J.Q. and Fujiyama, H. 1998. Importance of Na content and water status for growth in Na-salinized rice and tomato plants. *Soil Sci. Plant. Nutr*, 44: 197-208.

[14] Maxwell, D.P. and Bateman, D.F. 1967. Changes in the activities of some oxidases in extracts of Rhizoctonia infected bean hypocotyls in relation to lesion maturation. *Phytopathology*, 57: 132.

[15] Aebi, H. 1984. Catalase *in vitro*. *Metheds Enzymol.,* 105: 121-126.

[16] Robinson, S.P., Downton, W.J.S. and Milhouse, J.A. 1983. Photosynthesis and ion cintent of leaves and isolate chloroplasts of salt-stressed spinach. *Plant Physiol.,* 73: 238-242.

[17] Mackiny, G. 1941. Absorption of light by chlorophyll solution. *J. Biol. Chem.,* 140: 315-322.

[18] Khajeh-Hosseini M, Powell AA, Bimghan IJ (2003). The interaction between salinity stress and seed vigor during germination of soybean seeds. *Seed Sci. Technol.* 31: 715-725.

[19] Gomes Filho, E. and Scodek, L. 2002. Effect of salinity on ribonuclease activity of Vigna unguiculata cotyledons during germination. *J. plant Physilogy,* 132: 307-311.

[20] Elouaer MA, Hannachi C (2012). Seed priming to improve germination and seedling growth of sanflower (*Carthamus tinctorius*) under salt stress. *Eurasian J. Biosci.* 6: 76-84.

[21] Almodares, A., Hadi, M.R. and Dosti, B 2007. Effects of salt and stress on germination percentage and seedling growth in sweet sorghum cultivars. *J. Biological scinces.,* 7(8): 1492-1495.

[22] Taamalli, W., Abz, L., Youssef, N.B., Miled, D.D.B. and Zarrouk, M. 2004. Lipid breakdown in sunflower *(Helianthus annuus L.*) seeds during post germinative growth under salt-stress. *Rivista Italian delle Sostanze Grasse*, 81: 90-97.

[23] Ayaz, F.A. Kadioglu, A. and Turgut, R. 2000. Water stress effects on the content of low molecular weight carbohydrates and phenolic acids in *Ctenenthe setosa* (Rose). Eichler, *Can. J. Plant Sci.,* 80: 373-378.

[24] Greenway, H. 1963. Plant responses to saline substrates. Effect of nutrient concentration on the growth and ion uptake of Hordeum Vulgare during NaCl stress. *Aust. J. Biol. Sci.,* 16: 616-619.

[25] Hatung, W. 2004. Plant response to stress. Abscisic acid fluxes. Marcel Dekker. Inc., New York.

[26] Hawker, J.S,. and R.R. Walker, 1978. Effects of sodium chloride on expansion rates and invertase activity of leaves. *Aust. J. Plant Physiol.,* 5: 73-80.

[27] Schwarz, M. 1985. The use of saline water in hydroponics. *Soilless Cult.,* 1: 25-34.

[28] Yeo, A.R. 1983. Salinity resistance: physiologies and prices. *Physiol. Plant.,* 58: 214-222.

[29] Younis, M.E., El-Shahaby, O.A., Nemat Ally, M.M. and El-Bastawisy Zeinab, M. 2003. Kinetin alleviates the influence of water logging and salinity in Vigna sinensis and *Zea mays*. *Agronome,* 23: 277-285.

[30] Aboshama, H.M.S. and Hegazy, A.E. 2009. In vitro screening and production of salt tolerant *Critrus volkamariana* plant. *J. Agric. Sci. Mans. Univ.,* 34 (10): 10115-10133.

[31] Tripathi, S.B., Gurumurthi, K., Panigahi, A.K. and Shaw, B.P. 2007. Salinity induced changes in proline and betaine contents and synthesis in two aquatic macrophytes differing in salt tolerance. *Biol. Plant*, 51: 110-115.

[32] Hajibagheri, M.A., You, A.R., Flowers, T.J. and Collins, J.C. 1989. Salinity resistance in *Zea mays* fluxes of potassium, sodium and chloride, cytoplasmic concentrations and microsomal membrane lipids. *Plant cell and Env.,* 12: 753-757.

[33] Meloni, D.A., Gulotta, M.R. and Martinez, C.A. 2008. Salinity tolerance in Schinopsis quebracho Colorado. Seed germination, growth, ion relations and metabolic responses. *J. Arid Env.,* 72: 1785-1792.

[34] Munns, R., R.A. James and A. Lauchli, 2006. Approaches to increasing the salt tolerance of wheat and other cereals. *J. Exp. Bot*., 57: 1025-1043.

[35] Gechev, T.S., Breusegem, F.V., Stone, J.M., Denev., I. and Laloi, C. 2006. Reactive oxygen species as signals that modulate plant stress responses and programmed cell death. *Bio. Essays,* 28: 1091-1101.

[36] Jaleel, C.A., P. Manivannan, G.M.A. Lakshmanan, R. Sridharan and R. Panneerselvam, 2007. NaCl as a physiological modulator of proline metabolism and antioxidant potential in *Phyllanthus amarus*. *C. R. Biologies*, 330: 806-813.

[37] Mittova, V., M. Tal, M. Volokita and M.Guy, 2003. Upregulation of the leaf mitochondriral and peroxisomal antioxidative systems in response to salt induced oxidative stress in the wild salt tolerant tomato species *Lycopersicon pennelli*. *Plant Cell Env*., 26: 845-858.

[38] Ali, A.A. and Alqurainy, F. 2006. Activities of antioxidants in plants under environment stress. In: Motohashi, N. The lute in-prevention and treatment for diseases. India: Transworld research network, p. 187-256.

[39] Jiang, M.Y., 1999. Generation of OH and oxidation injury of plants under the condition of water stress. *Acta Bot. Sinica*., 41(3): 229-234.

[40] Turhan, E., L. Karni, H. Aktas, G. Deventurero, D.C. Chang, A. Bar-Tal and B. Aloni, 2006. Apoplastic anti-oxidants in pepper (*Capsicum annuum* L.) fruit and their relationship to blossom-end rot. *J. Hort.Sci. Biot*, 81(4): 661-667.

[41] Li, D., C. Li, H. Sun, W. Wang, L. Liu and Y. Zhang, 2010. Effects of drought on soluble protein content and protective enzyme system in cotton leaves. *Front. Agric. China*, 4: 56-62.

[42] Wang, W.B., Y.H. Kim, H.S. Lee, K.Y. Kim, X.P. Deng and S.S. Kwak, 2009. Analysis of antioxidant enzyme activity during germination of alfalfa under salt and drought stresses. *Plant Physiol. & Bioch*., 47: 570-577.

[43] Noreen, Z. and M. Ashraf, 2009. Assessment of variation in antioxidant defense system in salt treated pea (*Pisum sativum*) cultivars and it putative use as salinity tolerance markers. *J. Plant Physiol*., 166: 1764-1774.

[44] Hassanein, R.A., A.A. Hassanein, A.A. Haider and H.A. Hashem, 2009. Improving salt tolerance of *Zea mays* L. plants by presoaking their grains in glycine betaine. *Aus. J. Basic. Appl. Sci*., 3: 928-942.

[45] Gadalla, S.F. 2009. The roles of ascorbic acid α-tocopherol in minimize of salt-induced whert leaf senescence. J. Agric. Sci. Mans. Univ., 34 (11): 10645-10661.

[46] Lichtenthaler, HK. Langsdorf, G, Lenk S, and Bushman, C (2005). Chlorophyll fluorescence imaging of photosynthetic activity with the lamp fluorescence imaging system, phtosynthetica 43:355-369.

[47] Montesano, F and Van lersel MW (2007). Calcium can prevent toxic effects of Na^+ on tomato leaf photosynthesis but does not restore growth. *J Amer Soc Hort Sci* 132: 310-318.

[48] Afzal I, Butt A, Rehman, HU, Basra S and Afzal, A. 2012. Alleviation of salt stress in fine aromatic rice by seed priming. *Australian J Crop Sci* 6:1401-1407.

[49] Yeo, AR, lee KS. Lizard P. Boursier PJ, Flowers TJ. (1991). Short and long term effects of salinity on leaf growth in rice (*Oryza sativa*). *J Exp Bot* 42: 881-889.

[50] Parida, A.K., Das, A.B. and Mittra, B. 2004. Effects of salt on growth, ion accumulation, photosynthesis and leaf anatomy of the mangrove, *Bruguiera parviflora. Trees-Struct. Funct.*, 18: 167-174.

[51] Helaly, M.N., A.M. Salama and A.A. Arafa, 1984. Effects of salinity on growth mineral constituents, water fractions and endogenous growth substances in Horse bean plants. *J. Agric. Sci*. Mans. Univ., 9(2): 251-264.

[52] Mishra, S.N. and I. Sharma, 1994. Putrescine as growth inducer and nitrogen source for mustard seedling grown under salinity. *Int. J. Exp. Biol*., 32: 916-918.

[53] Pell, E.J. and M.S. Dann, 1991. Multiple stress-induced foliar senescence and implication for whole plant longevity. In. *Response of plants to multiple stresses*. (Eds. A.H. Mooney, V.E. Vinner, E.J. Pell and E. Chu), Academic Press Inc., pp. 189-204.

[54] Hashem, H.A., 2000. Molecular physiological studies on heat shock protein expression in a stressed plant. M.Sc.Thesis, Fac. Sci. Ain Shams Univ., Cairo, Egypt, (Thesis unpublished).

(Received 22 July 2014; accepted 02 August 2014)