Effect of chitosan on antioxidant enzyme activity, proline, and malondialdehyde content in *Triticum aestivum* L. and *Zea maize* L. under salt stress condition

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Abstract

Triticum aestivum L. and *Zea maize* L. are both sensitive to salinity stress which is a major problem faced by farmers today. In the present study, the effect of chitosan, a biologic elicitor under salinity stress was examined on growth parameters and biochemical markers in maize and wheat seedlings. Seeds of wheat and maize plants were coated with chitosan 25%, 50%, and 75% solutions before they were planted and subjected to 0, 50, 100, 150, and 200 mM salinity stress under a 11/8 h photoperiod and at 25±2 °C temperature condition during 7 days. Then, the growth parameters including germination percentage, root and shoot growth as well as seedling weight were recorded. The biochemical markers including catalase and peroxidase activity and malondialdehyde, proline, and protein contents were measured at day seven of the experiment. Significant difference in relation with growth parameters was observed at high concentrations of chitosan in comparison with the control plants under salt stress. Catalase and peroxidase activity and protein content increased under salinity stress and chitosan at high concentration reduced catalase and peroxidase activity. Salinity stress induced lipid peroxidation and malondialdehyde accumulation while chitosan reduced malondialdehyde content of the plants under salinity stress. The synthesis of protein was significantly increased with increasing the chitosan concentration. Generally, the growth parameters of both seedlings were improved and unfavorable effects of salinity were reduced when the seeds were coated with chitosan. Application of chitosan at low concentrations increased antioxidant enzyme activity and proline content and decreased MDA accumulation. In conclusion, chitosan at an appropriate dose improved growth performance and biochemical marker fluctuation under salinity stress.

Keywords: chitosan; salinity; maize; wheat; antioxidant enzymes; proline; malondialdehyde; growth

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Introduction

Biotic and abiotic stresses are limiting factors that pose challenges for making use of water of soil in agricultural practices. One of the most important stressors which limit crop production is salinity (Bohnert et al., 1995, and Hasanuzzaman et al., 2013, Shtereva et al., 2015, Zayed et al., 2017). Salinity is the most important detrimental factor for agricultural production in arid and semiarid regions of the world negatively affecting all aspects of plant growth and performance (Konuşkan et al. 2017). Shortage in the available

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water for plants under salinity stress leads to poisoning due to toxic ions, reduced plant performance, nutritional abnormalities, impaired growth, and reduced quality of the crop (Bernstein et al., 1993; Bohnert et al., 1995, Patel et al., 2010).

Elicitors are chemicals or various biologic factors that can induce physiological changes in plants (Zhao et al., 2005). One of the methods of mitigating the negative effects of abiotic stresses in many plants and improving their quality and performance is employing biologic stimulants. Several compounds such as chitosan have been identified with eliciting properties which stimulate plants reactions to stresses and their defense mechanisms (Bautista-Baños et al., 2006). Chitosan, which is produced from deacetylation of kitin, is a biodegradable compound made of crustaceans' shells, cell wall of fungi, cuticles of insects and some algae and the second most prevalent biopolymer in the nature after cellulose (Uthairatanakij et al., 2007). This compound with its unique biological and physiological properties was used in various pharmaceutical and agricultural industries (Cuero et al., 1991, Lizárraga-Paulín et al., 2011).

Chitosan has been confirmed to control numerous fungal, bacterial, and viral diseases on various horticultural crops (Bautista-Baños et al., 2006; Liu et al., 2007). Chitosan controlled infection of Fusarium in wheat and increased seedlings' resistance by accumulation of phenolics and lignin compounds (Bhaskara et al., 1999).

The stimulation effect of chitosan on germination of crops is already established (Mandal, 2010; Uthairatanakij et al. 2007; Lizárraga-Paulín et al., 2011; Zayed et al., 2017; Choudhary et al., 2017). Application of chitosan to improve growth indexes of crops are studied e.g., chitosan increased stem length, leaf number, length and area, and flowering and number of flowers in Gerbera (Wanichpongpan et al., 2001). Chitosan also was reported to stimulate growth in various plants e.g., soybean (Lee et al., 2005).

Wheat (*Triticum aestivum* L.) and maize (*Zea maize* L.) are both monocotyledon plants, belonging to the Poaceae family. These nutritious grains are the most important cereals in the worlds and a large portion of the world population depends on them as the main source of food (Konuşkan et al., 2017). The consumption of wheat and maize per capita in Iran is also high and their production is particularly important. Due to the sensitivity of these crops to salinity stress (Shtereva et al., 2015), their growth and performance is highly affected by salt and reduced yield.

Regarding the increasing trend of salinity in Iran and its potential threats for the crops and also considering the significance of wheat and maize for human food, the present study was carried out to examine the mitigation role of chitosan on the adverse effects of salt stress and its potential to improve plant growth under salinity.

Materials and Methods

Seed germination was done on Petri dishes with lid and two filter papers and 10 seeds were put in each dishes. The dishes were then kept in an incubator set at 25±2 °C and 16/8 h photoperiod for 7 days.

Salinity stress was applied at 50, 100, 150, and 200 mM concentrations. In order to apply chitosan treatment, wheat and maize seeds were soaked in 25%, 50%, 75% concentrations of chitosan dissolved in acetic acid 1%. for 3 hours. Chitosan powder was purchased from Sigma Aldrich Company, Germany. Then, the treated seeds were rinsed in distilled water and dried for 24 hours. Then the seed were irrigated with different levels of salinity solution every day. The number of germinated seeds in each dish was counted every day and also root and shoot lengths were measured to compare with the control. Pincers and a sterile measurement ruler were used to record seedling lengths (root and shoot lengths) under laminar hood on days 3, 5, and 7.

After 7 days of treatment, the seedlings were harvested, rinsed 3 times with distilled water to remove salt residue, and kept at -80 °C to study antioxidant enzyme activities and other biochemical factors such as proline, protein and malondialdehyde.

Proline assay

Proline content was measured using the method of Bates et al. (1973). Briefly, 10 ml of sulfosalicylic acid 3% was added to 100 mg plant dry matter. After 24 hours, the solution was centrifuged 1300 rpm for 10 minutes. Two ml ninhydrin was added to 2 ml of the supernatant. Then 1 ml glacial acetic acid was added to the solution and the test tubes were kept in boiling water of Ben Mary for 1 hour. Four ml of toluene was added to each tube after they were cooled. This resulted in two phases of the solution. The top phase was subjected to spectrophotometry analysis (Specord 210 model) and the absorption was recorded at 520 nm.

Malondialdehyde assay

Malondialdehyde (MDA) content was measured using the method described by Ohkawa et al. (1979). Plant tissues including stems and roots (0.2 g) were cut into small pieces and homogenized in 2 ml 3-chloro acetic acid 5% in the vicinity of ice using a homogenizer. They were then centrifuged at 12000 rpm for 15 minutes and the supernatant was removed. 0.5 ml of the solution was then mixed with the same volume of thiobarbituric acid and 3-chloro acetic acid 20% before it was incubated at 96 °C for 25 minutes. Absorbance of the supernatant was recorded spectrophotometrically at 532 nm. Thiobarbituric acid and 3-chloro acetic acid 20% were used as control. MDA content was measured using the standard curve.

Extraction of peroxidase and catalase

Seven-day-old seedlings (1 g) were ground in 5 ml NaCl solution (5%) and then homogenized. It was then centrifuged at 1000 rpm for 8 minutes. The supernatant was removed and kept at 4 °C for assaying catalase and peroxidase activities.

Catalase and peroxidase assay

The activity of catalase was measured based on decomposition of H_2O_2 using the method of Aebi et al. (1984). The prepared tissue extract was added to the reaction solution containing 100 mM potassium phosphate buffer and 15 mM H_2O_2. Absorbance was then recorded at 240 nm using the spectrophotometer.

The activity of peroxidase was measured using the method of Foyer and Halliwell (1976). The solution contained 1 mM potassium phosphate (pH: 7.5) in 0.36 mM EDTA and 9.9 mM isoascorbate. The reaction solution containing 200 mM sodium phosphate buffer (pH: 7.5), 0.1 mM NADPH, 0.25 mM glutathione, 1.5 mM $MgCl_2$, 0.2 mM EDTA, and 100 µl of the enzymatic extract were added and incubated at 37 °C for 1 minute. The absorbance was then read at 340 nm.

Protein assay

Shoot protein content was measured using the automatic nitrogen measurement equipment Kjeltec™ 2300 Unit (FOSS, Denmark). 1 g of each sample was weighed using a digital scale (0.0001 precision) and fed into the digestion tube of Kjeltec Analyzer together with 5 g catalyst and 20 ml sulfuric acid. After 2 hours when the digestion was done and the tubes were cooled, the tubes containing digested matter were put in distillation equipment. After distillation, titration was done using the automatic Kjeltec equipment. Protein contents of the samples were assayed using the method of AOAC (2002).

Data Analysis

A factorial study was conducted based on a completely randomized design with 6 replications. Data were submitted to statistical analysis using SPSS and Duncan's multiple range test as the statistical test. Graphs were drawn using Excel 2010.

Results

Effects of salt stress, chitosan, and their interaction on shoot and root lengths as well as seedling weights of maize and wheat are shown in Figs. I and II, respectively. Shoot length in maize increased when 50 mM NaCl was applied while high concentration of salt decreased this

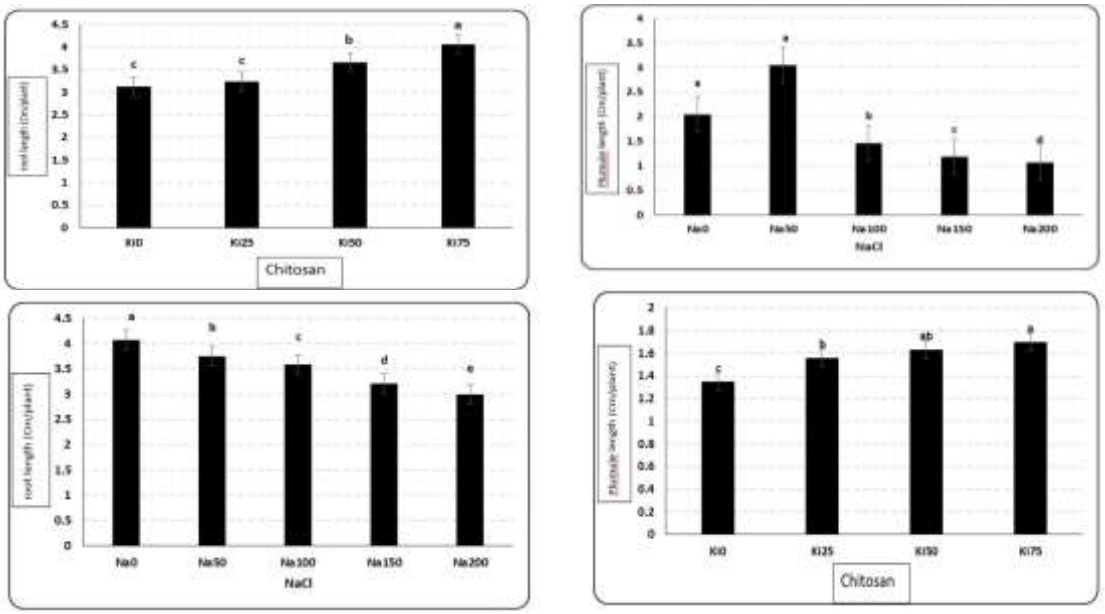

Fig. I. Effect of salt stress and chitosan on shoot and root length in maize.

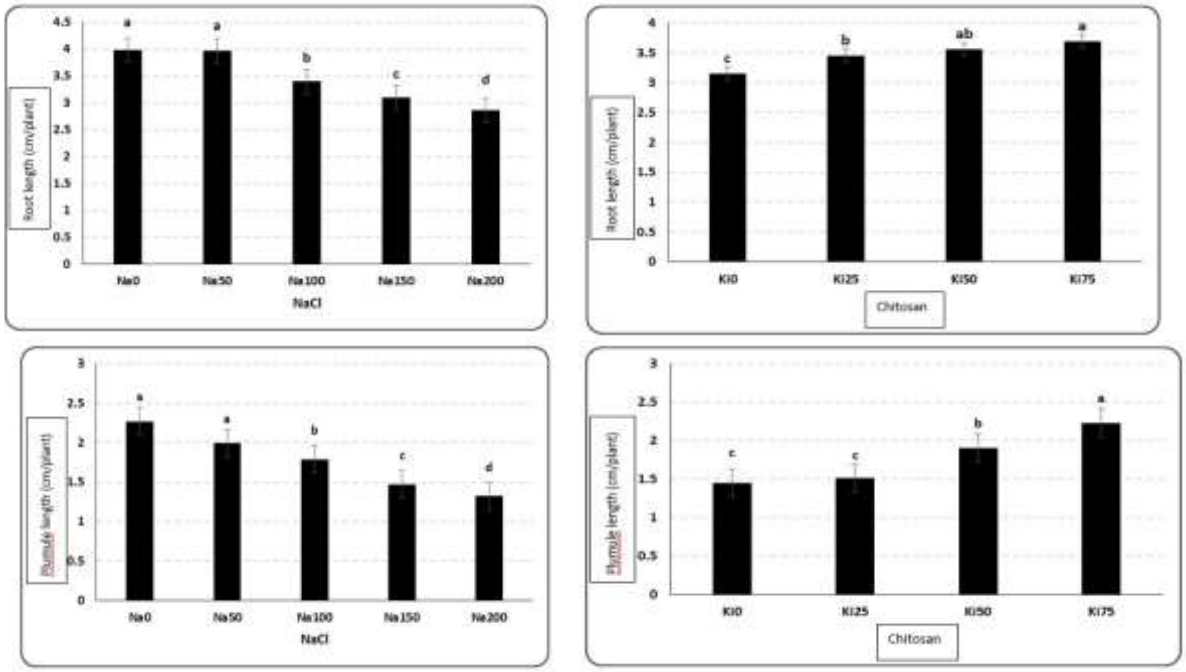

Fig. II. Effect of salt stress and chitosan on shoot and root length in wheat.

parameter. On the other hand, shoot length of wheat decreased by increasing salt concentration. Salt stress also decreased root length in both plants. On the other hand, application of chitosan increased shoot and root length in both plants and the highest shoot and root length were observed at the highest concentration of chitosan (75%).

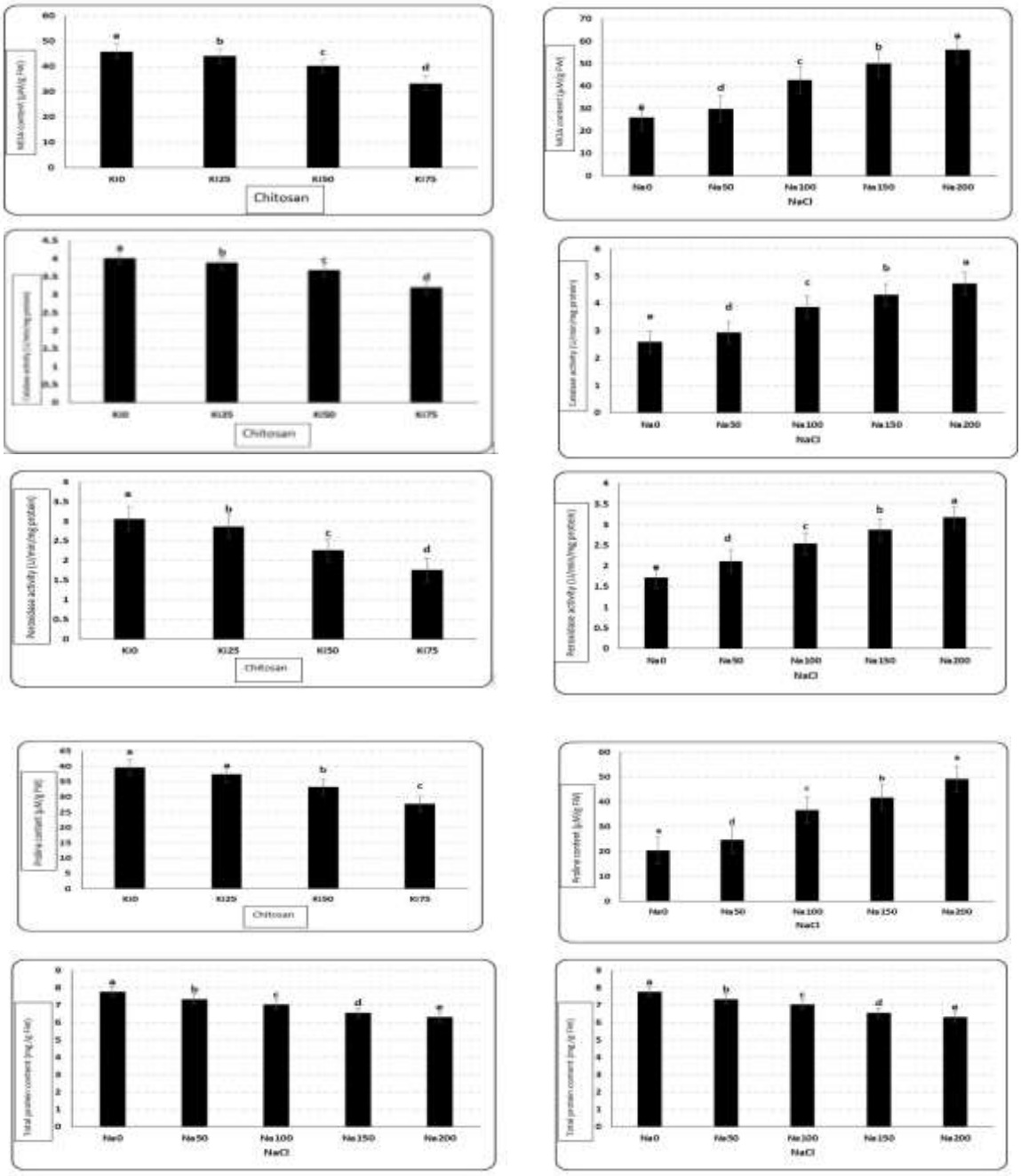

Fig. III. Effect of salt stress and chitosan treatment on catalase and peroxidase activity, MDA, protein and proline content in maize seedlings

Seedling weight increased when 50 mM NaCl concentration was used while high concentration of NaCl inhibited growth and decreased seedling weight. Application of chitosan increased seedling weight gradually and the highest seedling weight was observed at high concentrations of chitosan in both plants. The interaction effect of salinity and chitosan on shoot length of maize plants showed that the highest shoot length was obtained under 50 mM concentration of NaCl and 25% and 50% of chitosan. Maximum root length was observed at 75% chitosan and 50 mM concentration of NaCl and under control condition. Moreover, the highest seedling weight was found at 50 and 200 mM concentration of NaCl as well as 50% and 75% chitosan treatment. Interaction of salinity and chitosan on shoot length of wheat showed that the maximum shoot length was observed at 75% chitosan under control condition and 50 mM

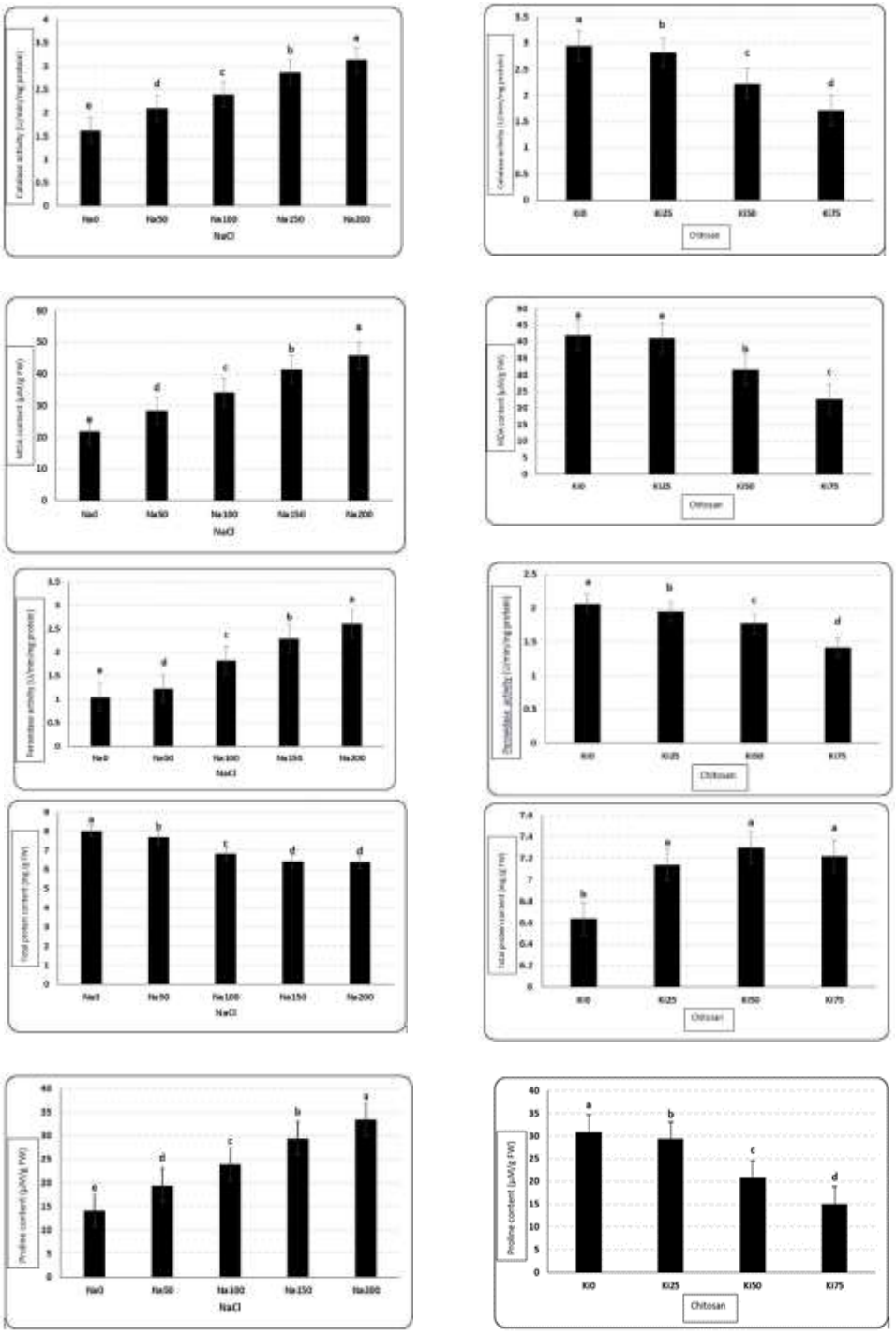

Fig. IV. Effect of salt stress and chitosan treatment on catalase and peroxidase activity, MDA, protein, and proline contents in wheat seedlings

concentration of NaCl. The highest root length was found at 75% chitosan treatment under control condition. Also, the maximum seedling weight was found at 50 mM concentration of NaCl together with 50% chitosan.

Effects of salt stress, chitosan, and their interaction on catalase, peroxidase, MDA, protein, and proline contents of maize and wheat seedlings are shown in Figs. (III) and (IV), respectively. Chitosan had a positive effect on growth parameters of maize seedlings. Catalase activity

increased under salinity stress and maximum catalase activity was found at 200 mM concentration of NaCl. Chitosan at high concentration had no positive effect on catalase activity. The highest catalase activity was found at 200 mM concentration of NaCl and the treatment with chitosan 25%. Peroxidase activity increased under salinity stress and application of chitosan reduced peroxidase activity. The highest peroxidase activity was recorded at 200 mM NaCl concentration without chitosan treatment. Moreover, salinity stress promoted MDA production in maize seedlings. On the other hand, chitosan application had no effect on MDA accumulation and the highest MDA was observed at 200 mM concentration of NaCl and under 0 and 25% of chitosan application. Salinity stress also lead to the accumulation of proline while high concentration of chitosan decreased proline accumulation. The highest proline content was seen at 200 mM concentration of NaCl and 25 % chitosan and no chitosan application.

In wheat seedlings, catalase activity was enhanced under salinity stress and chitosan at high concentration decreased catalase activity. The highest catalase activity was found at 200 mM concentration of NaCl without chitosan treatment. Peroxidase activity increased under salinity stress while chitosan at high concentration decreased peroxidase activity. The highest peroxidase activity was found at 200 mM concentration of NaCl without chitosan treatment. Besides, MDA concentration increased in wheat seedlings under salinity stress while using chitosan decreased MDA content. The maximum MDA content was observed at 200 mM concentration of NaCl without chitosan treatment. Finally, proline accumulation increased gradually under salinity stress whereas chitosan at high concentration decreased proline content. The maximum proline content was seen at 200 mM concentration of NaCl without chitosan treatment.

Discussion

Findings of the study suggest that salt stress decreased root length in both plants. On the other hand, while shoot length in maize increased under 50 mM NaCl, high concentration of salt decreased shoot length. Reduction of shoot and root length especially at high levels of salinity was also reported in maize genotypes (Shtereva et al., 2015; Konuşkan et al., 2017). In fact, salt stress induces osmotic stress, ion toxicity, and also oxidative stress (Kaya et al., 2013).

Treatment with chitosan increased shoot and root length in both plants and the highest shoot and root lengths were recorded under the highest concentration of chitosan (75%). These findings are in agreement with those of Ma et al. (2012) and Lizárraga-Paulín et al. (2011) in wheat and corn seedlings, respectively. Studies have shown that using elicitors such as chitosan enhances shoot and root heights as well as shoot and root dry weights in the case of maize (Guan et al., 2009 and Lizárraga-Paulín et al., 2011), rice (Ruan and Xue, 2002 and Boonlertnirun et al., 2008), and beans (*Phaseolus vulgaris* L) (Zayed et al., 2017) and improves tolerance of seedlings to stress conditions.

Seedling weight increased when 50 mM Nacl concentration was used though high concentration of NaCl inhibited growth and decreased seedling weight. Similar results in relation to shoot and root dry weight values were reported by Konuşkan et al. (2017).

Chitosan improved shoot and root length as well as seedling weight in wheat seedling under salinity stress. Studies show that chitosan increases the growth rate of root and shoot of radish (*Raphanus sativus* L.) (Tsugita et al. 1993), soybean (Lee et al. 2005), and sweet basil (Kim, 2005).

In both maize and wheat seedlings, chitosan at low concentrations accelerated catalase and peroxidase activity and at high dose decreased catalase and peroxidase activity. Exogenous application of chitosan was reported to increase catalase and peroxidase in eggplants (Mandal, 2010) and tomatos (Ortega-Ortiz et al., 2007). According to Zayed et al. (2017) the highest catalase and peroxidase activities were found under application of 1% and 3% nano-chitosan concentration, respectively. Catalase activity in leaf and root tissue of Lens culinaris M. did not change under salinity stress (Bandeoglu et al., 2004). Therefore, the variation in catalase and peroxidase activity seems to be related to plant

species, stress condition, as well as type and concentration of elicitors.

Zayed et al. (2017) reported that proline accumulation increased under 50 mM Nacl concentration and application of 0.1 % chitosan. Salinity stress also increased proline and MDA content in safflower (*Carthamus tinctorius* L.) and sunflower (*Helianthus annuus* L.) and low concentrations of chitosan reduced MDA and proline production (Jabeen and Ahmad, 2013). In wheat seedlings, oligo chitosan pretreatment reduced MDA content in leaves and accumulation of proline was accelerated (Ma et al., 2012). Application of chitosan at low concentrations in *Carthamus tinctorius* L. under salinity stress enhanced proline accumulation and reduced MDA content (Mahdavi et al., 2013). The accumulation of osmolytes such as proline is an adaptive mechanism in plants against salt stress conditions that increases tolerance of most species to stress conditions (Hasanuzzaman et al., 2013). Under salinity stress, MDA content increases because of lipid peroxidation (Quartacci et al., 2000) while application of chitosan decreases MDA content. Chitosan can reduce lipids oxidation through chelating with ions or combination with lipids (Sui et al., 2002).

Both in maize and wheat seedlings, salinity decreased protein content. On the other hand, while chitosan increased protein content of maize seedlings, applying chitosan had no effect on accumulation of protein. Studies have shown that chitosan plays a regulating role in biosynthesis of substances including protein (Jiang et al., 2006 and Jin et al., 2006). Also, protein content in both plants reduced as a result of increase in salinity stress which shows the damaging effect of salinity on plants' total protein content.

References

Aebi, H. 1984.Catalase. In: Packer, L. ed. Methods in enzymol. Academic press, Orlando., 105: 121-126.

AOAC. 2002.International methods committee guidelines for validation of qualitative and quantitative food microbiological official methods of analysis. *Journal of Aoac International* 85(5): 1187-1200.

Bhaskara MV, J. Arul, P. Angers and **L. Couture**. 1999. 'Chitosan treatment of wheat seeds induces resistance to *Fusarium graminearum* and improves seed quality'. *J Agric Food Chem.* 47(3):1208-1216.

Bandeoglu E. ,F. Eyidogan, M. Yucel and **H. A. Oktem.** 2004. 'Antioxidant response of shoots and roots of lentil to NaCl Salinity stress. Plant Growth Regul, 42 (1): 69-77.

Bates L.S., R. P. Waldren and **I. D. Teare**. 1973. 'Rapid determination of free proline for water stress studies'. Plant and Soil, 39 (1): 205-207.

Bautista-Baños, S., A. N. Hernández-Lauzardo and **M. G.Velázquez-del Valle**.2006. 'Chitosan as a potential natural compound to control pre and postharvest diseases of horticultural commodities'. *Crop Protection*. 25: 108-118.

Bernstein, N.,W. K. Silk and **A. L. Lauchli**. 1993. 'Growth and development of sorghum leaves under conditions of NaCl stress'. *Planta*, 191 (4):433-439.

Bohnert, H.J., D. E. Nelson and **R. G. Jensen**. 1995. 'Adaptation to environmental stresses'. *The Plant Cell*, 7: 1099–1111.

Boonlertnirun S, C. Boonraung and **R. Suvanasara** .2008. Application of chitosan in rice production. *JOM-J Min. Met. Mat. S.* 18: 47-52.

Cuero R.G., G. Osuji and **A. Washington**. 1991. 'N-carboxymethyl chitosan inhibition of aflatoxin production: role of zinc'. *Biotechnology letters*, 13 (6): 441-444.

Foyer, C. H. and **B. Halliwell**. 1976. 'The presence of glutathione and glutathione reductase in chloroplast: a proposed role in ascorbic acid metabolism'. *Planta* ,133: 21-25.

Guan YJ, J. Hu, X. J. Wang and **C. X. Shao**. 2009. 'Seed priming with chitosan improves maize germination and seedling growth in relation to physiological changes under low temperature stress'. *J. Zhejiang Univ. Sci. B.,* 10: 427-433.

Hasanuzzaman M., K. Nahar and **M. Fujita**. 2013. 'Plant response to salt stress and role of exogenous protectants to mitigate salt-induced damages'. In: P. Ahmad et al. (eds.),

Ecophysiology and Responses of Plants under Salt Stress, DOI 10.1007/978-1-4614-4747-4_2, © Springer Science+Business Media, LLC.

Jabeen, N. and **R. Ahmad.** 2013. 'The activity of antioxidant enzymes in response to salt stress in safflower (*Carthamus tinctorius* L.) and sunflower (*Helianthus annuus* L.) seedlings raised from seed treated with chitosan'. *Journal of the Science of Food and Agriculture*, 93(7): 1699-1705.

Jiang, L., L. Duan, X. Tian, B. Wang, H. Zhang, M. Zhang and **Z. Li**. 2006. 'NaCl salinity stress decreased *Bacillus thuringiensis* (Bt) protein content of transgenic Bt cotton (*Gossypium hirsutum* L.) seedlings'. *Enviromental and Experimental Botany*, 55 (3): 315-320.

Jin, J., Sh. Ningwei., B. Jinhe and **G. Junping**. 2006. 'Regulation of ascorbate peroxidase at the transcript level is involved in tolerance to postharvest water deficit stress in the cut Rose (*Rose hybrida* L.)'. *Postharvest Biology and Technology*, 40 (3): 236-243.

Kaya C.,O. Sonmeza, S. Aydemira, M. Ashraf and **M. Dikilita**. 2013. 'Exogenous application of mannitol and thiourea regulates plant growth and oxidative stress responses in salt-stressed maize (*Zea mays* L.)'. *Journal of Plant Interactions*, 8 (3): 234-241.

Kim, H. J. 2005. 'Characterization of bioactive compounds in essential oils, fermented anchovy sauce, and edible plants, and, induction of phytochemicals from edible plants using methyl jasmonate (MeJA) and chitosan'. Archived Dissertations. 728.https://tigerprints.clemson.edu/arv_dissertations/728.

Konuşkan Ö., H. Gözübenli , I. Atiş and **M. Atak**. 2017.' Effects of salinity stress on emergence and seedling growth parameters of some maize genotypes (*Zea mays* L.)'. *Turkish Journal of Agriculture - Food Science and Technology*, 5(12): 1668-1672.

Lee, Y. S., Y. H. Kim and **S. B. Kim.** 2005. 'Changes in the respiration, growth, and vitamin C content of soybean sprouts in response to chitosan of different molecular weights'. *Horticulture Science,* 40: 1333-1335.

Ma, L., Y. Li, C. Yu, Y. Wang, X. Li, N. Li, Q. Chen and **N. Bu.** 2012. 'Alleviation of exogenous oligochitosan on wheat seedlings growth under salt stress'. *Protoplasma*, 249: 393-399.

Liu, J., S. Tian, X. Meng and **Y. Xu.** 2007. 'Effects of chitosan on control of postharvest diseases and physiological responses of tomato fruit'. *Postharvest Biology and Technology*, 44: 300-306.

Lizárraga-Paulín E. G., I. Torres-Pacheco, E. Moreno-Martínez and **S. Patricia Miranda**. 2011. 'Chitosan application in maize (*Zea mays*) to counteract the effects of abiotic stress at seedling level'. *African Journal of Biotechnology*, 10(34): 6439-6446.

Mahdavi, B. S. A. M. Modarres Sanavy, M. Aghaalikhani and **M. Sharifi**. 2011. 'Chitosan improves osmotic potential tolerance in safflower (*Carthamus tinctorius* L.) seedlings. *Journal of Crop Improvement*, 25(6): 728-741.

Mandal, S. 2010.' Induction of phenolics, lignin and key defense enzymes in eggplant (*Solanum melongena* L.) roots in response to elicitors'. *African Journal of Biotechnology*, 9: 8038-8047.

Ortega-Ortiz H, A. Benavides-Mendoza,R. Mendoza-Villarreal,H. RamírezRodríguez and **K.D.A. Romenus KDA** .2007.' Enzymatic activity in tomato fruits as a response to chemical elicitors'. *J. Mex. Chem. Soc.* 51: 141-144.

Ohkawa, H., N. Ohishi and **Y. Yagi**. 1979. 'Assay of lipid peroxides in tissues by thiobarbituric acid reaction'. *Ann. Biochem*., 5: 51-358.

Ruan S.L. and **Q. Z. Xue**. 2002. 'Effects of chitosan coating on seed germination and salt tolerance of seedlings in hybrid rice (*Oryza sativa* L.)'. *Acta Agron. Sinica.* 28: 803-808.

Patel, P. R.,S. S. Kajal, S. S., Patel, V. R., Patel and **Khristi S. M.** 2010.' Impact of salt stress on nutrient uptake and growth of cowpea'. *Brazilian Journal of Plant Physiology*, 22 (1): 43-48.

Quartacci M. F., C. Pinzino, C. L. M. Sgherri, F. D. Vecchia and **F. Navari Izzo**. 2000. 'In excess copper induce changes in lipid composition and fluding of psll enriched membranes in wheat'. *Physiologia Plantarum*, 108 (1): 87-93.

Tsugita T,K. Takahashi, T. Muraoka and **H. Fukui** . 1993. 'The application of chitin/chitosan for

agriculture'. 7th Symposium on Chitin and Chitosan, Fukui, Japan, pp 21-22 (in Japanese)

Uthairatanakij, A., J. A. Teixeira da Silva and **K. Obsuwan**. 2007. 'Chitosan for improving orchid production and quality'.*Orchid Science and Biotechnology* ,1: 1-5.

Wanichpongpan, P., K. Suriyachan and **S. Chandrkrachang.** 2001. 'Effect of Chitosan on the growth of Gerbera flower plant (*Gerbera jamesonii*)'. P. 198-201. In: T. Uragami et al. (ed.) Chitin and Chitosan in Life Science. Yamaguchi. Japan.

Shtereva L. A., R. D. Vassilevska -Ivanova and **T. V. Karceva**. 2015. 'Effect of salt stress on some sweet corn (*Zea mays* L. var saccharata) genotypes'. *Arch. Biol. Sci., Belgrade*. 67(3):993-1000.

Sui, X Y, W. Q. Zhang W. Xia and **Q. Wang**. 2002. 'Effect of chitosan as seed coating on seed germination and seedling growth and several physiological and biochemical indexes in rapeseed'.*Plant Physiology Communications*, 38: 225-227.

Zayed M. M., S. H. Elkafafi, A. M. G. Zedan and **S. F. M. Dawoud.** 2017. 'Effect of nano chitosan on growth, physiological and biochemical parameters of *Phaseolus vulgaris* under salt stress'. *Journal of Plant Production*, 8 (5): 577-585.

Zhao, J., L. C. Davis and **R. Verpoorte**. 2005. 'Elicitor signal transduction leading to production of plant secondary metabolites'. *Biotechnology Advance*, 23 (4): 283-333.