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# Effects of salicylic acid on physiological traits of myrtle seedlings in salt stress condition

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**Abstract**. Salicylic acid is known as an important signaling molecule that regulates plant reactions to environmental stresses. It plays a critical role in the regulation of physiological functions as non-enzymatic antioxidant. In this study we investigated the effects of different concentrations of salicylic acid (0, 50, 100 and 150 ppm) that were sprayed on one age seedlings of myrtle (*Myrtus communis* L.) with different levels of NaCl (0, 25, 50, and 100 mM) on activities of superoxide dismutase (SOD), guaiacol peroxidase (GPX), ascorbate peroxidase (APX), catalase (CAT), glycinebetaine (GB), proline, hydrogen peroxide ($H_2O_2$) and ionic leakage (ELI). Interactions of salicylic acid at 50 ppm with 50 mM salinity showed significantly increase in the CAT activity. GPX and APX were significantly higher in 50 mM salinity level with 50 and 100 ppm salicylic acid concentrations. The highest activity of SOD was observed in 50 mM of salt and 50 ppm of salicylic acid, indicated that 50 ppm had highest resistance against salt concentration. Proline in 50 mM salinity with 50 ppm salicylic acid showed the most significant increase in the 100 mM of salinity had a sharp decline in their interactions. GB had maximum amount in 25 mM salinity with all of salicylic acid treatments. Ionic leakage had significant interactions but was not significant with increasing salinity and salicylic acid treatments. The hydrogen peroxide showed the most significant increase in 100 mM salinity with 100 and 150 ppm salicylic acid levels respectively, and effectiveness of their interaction in 100 mM of salt with all treatments of salicylic acid except control.
**Key Words**: *Myrtus communis*, salinity, plant growth, salicylic acid.

**Introduction**. Soil salinity and alkalinity are main problems in almost all of irrigated areas and might be occur in agricultural fields and grasslands. In other words, all lands are capable to be salted. Soil salinity is one of the most serious problem attracts some scientists to overcome this obstruction by improving salt tolerant lines (Gholizadeh & Navabpour 2011).

Salinity stress reduces the plant growth and because of interruption in equivalence of essential elements absorption, water and oxidative tension, decrease the yield (Parida & Das 2005; Mollassiotis et al 2006). Stress induced reactive oxygen species (ROS) accumulation is counteracted by enzymatic antioxidant systems that include a variety of scavengers, such as superoxide dismutase (SOD), guaiacol peroxidase (GPX), ascorbate peroxidase (APX), catalase (CAT), and non-anzymatic low molecular metabolites, such as proline and sugar (Sarvajeet & Narendra 2010). The researches about impact of different tensions on grassland and forest plants are less than other fields in agriculture (Winicow 1998). Less proficiency of products such as subordinate forestrial products, may be due to abiotic stresses (Shanker & Venkateswarlu 2011). One of the physiologic methods that in recent years, had been used for diminishing the environmental stresses on different plants, is application of tension extenuating materials (Yuan & Lin 2008). One of these materials is salicylic acid that is an important messenger molecule and it helps plant to response to the environmental tensions, and has a consequential role in adjustment of physiological activities in plant, such as a non-enzymatic antioxidant (Arfan et al 2007). Exogenous salicylic acid alters the activities of antioxidant enzymes and increases plant tolerance to abiotic stress by decreasing generation of ROS. It has been found that salicylic acid has different effects on stress adaptation and damage development of plant that depend on plant species, concentration, method and time of salicylic acid application (Metwally et al 2003). Salicylic acid has obtained particular attention because of inducing protective effects on plants unde NaCl salinity (Simaei et al 2011). Several studies have shown that the effect of cytotoxicity induced by salt stress can be ameliorated by the exogenous application of salicylic acid (Simaei et al 2012).

Myrtle (*Myrtus communis* L.) is an evergreen shrub that reaches a height of 3 meters. Myrtle has a considerable importance, given the role in semi-arid ecosystems in terms of soil conservation and prevent soil erosion and also its importance to use in multipurpose culture. Myrtle is a compatible plant in sub-humid areas of Iranian provinces included: Lorestan, Fars, Kerman, Sistan and Isfahan (Azadbakht 1999; Zargari 1996). The objectives of this work were to study the effect of salinity stress on the activities of antioxidant enzymes in myrtle and to examine whether the harmful effects of salinity stress can be offset by the exogenous application of salicylic acid.

**Material and Method**. In this study, seeds were collected from maternal plants growing in Jiroft, Kerman province of Iran. Pots were in green house of agricultural research and natural resources center of Kerman. During the emergence of seedlings, the weak seedlings were removed to have a suitable room in each pot.

This study was done using a factorial experiment in a randomized complete block design with four replications. Factors were included: four levels of salinity (0, 25, 50 and 100 mM) and four levels of salicylic acid (0, 50, 100 and 150 ppm).

Pot experiment done by 20-centimeter vases that were filled by sand, peat and field soil (proportion was 1:1:1). At the age of one year, salicylic acid was sprayed to pots at levels of: 0, 50, 100 and 150 ppm then salinity treatments at amounts of: 0, 25, 50 and 100 mM of sodium chloride were applied. The plants were irrigated by 3:1 Hoagland solution two times per week for two months and salicylic acid was sprayed on the plants every two weeks. Sodium chloride was added to 3:1 Hoagland solution (Tattini et al 2006). The amount of Glycinebetaine (GB) in dried leaves (third leaf from plant top) was measured (Grieve & Grattan 1983) and at a wavelength of 365 nm was read. Measurement of prolin was done via spectrophotometry at $25\pm1$ºC (Bates et al 1973) and at a wavelength of 520 nm was read. To measure the electrical conductivity of damage to membrane, ion leakage was assessed (Campos et al 2003).

***Measurement of enzyme activity****.* CAT activity was measured by the destruction of $H_2O_2$ by CAT (Aebi 1984) and at a wavelength of 240 nm was read. APX activity was evaluated by method of Ranieri et al (2003) and at a wavelength of 290 nm was read. Mensuration of GPX activity was done by method of Cakmak & Marschner (1988) and at a wavelength of 470 nm was read. Measuring the activity of superoxide dismutase was done photometric by method of Dhindsa et al (1981) and at a wavelength of 560 nm was read. The content of hydrogen peroxide was measured based on Loreto & Velikova (2001) and at a wavelength of 390 nm was read.

***Statistical calculations****.* Analysis of variance was done by SAS (ver. 9.1) and the comparison of means was carried out using Duncan's multiple range test.

**Results and Discussion**. Table of analysis of variance (Table 1) showed that in this experiment salinity and salicylic acid treatments and interaction between salinity and salicylic acid at level of 1% for CAT were significant, the highest level of CAT enzyme was observed at 50 mM of salinity and then 25, 0 and 100 mM respectively. CAT enzyme activity at level of 25 and 50 mM of salinity, was more than control treatment, while this enzyme, only in control plants (at level of 0 salinity) there was more activity compared to 100 mM. There was a diminish in CAT enzyme activity at level of 100 mM that this is injurious to plant, because this decrease compared to control plants was significant. Assessing the main effect of salicylic acid showed that salicylic acid at 50 ppm improved CAT enzyme activity but it had a decrease trend at 100, 0 and 150 ppm respectively. The least CAT enzyme activity was observed at 150 ppm that this decline was significant.

Evaluation of interactions showed that at 100 mM salinity and 150 ppm salicylic acid, CAT enzyme had the least activity but the highest activity was observed at 50 mM salinity and 50 ppm salicylic acid (Figure 1).

Table 1

Analysis of variance mean squares of myrtle traits

| *Source* | *Df* | *CAT* | *GPX* | *SOD* | *APX* | *$H_2O_2$* | *ELI* | *GB* | *Proline* |
|---|---|---|---|---|---|---|---|---|---|
| Replication | 3 | 1.98 | 0.06 | 8.5 | 0.07 | 151.18 | 14.51 | 2.24 | 0.72 |
| S | 3 | *26.34 | *0.98 | *185.38 | *1.65 | *49195.85 | *2094.25 | *304.35 | *18.34 |
| SA | 3 | *52.4 | *0.65 | *90.34 | *1.42 | *10474.58 | 52.41 | *160.1 | *27.04 |
| S*SA | 9 | 4.95 | *0.18 | *27.1 | *0.2 | *4113.01 | 28.59 | 17.25 | *2.81 |
| Error | 3 | 2.41 | 0.04 | 7.83 | 0.05 | 1066.92 | 28.62 | 14.95 | 0.87 |
| CV | 45 | 20.97 | 25.87 | 25.72 | 29.55 | 13.42 | 24.14 | 25.3 | 22.22 |

$p < 0.01$ - *distinctly significant, S - salinity, SA - salicylic acid; Df - degrees of freedom; CAT - catalase; GPX - guaiacol peroxidase; SOD - superoxide dismutase; APX - ascorbate peroxidase; $H_2O_2$ - hydrogen peroxide; ELI - ionic leakage; GB - glycinebetaine.

GPX and APX enzymes showed same trend to different treatments. Table of analysis of variance showed that salinity and salicylic acid treatments and interaction between these two treatments showed a significant effect at probability 1%. The highest GPX and APX activity was at 50 mM salinity then at 25, 0 and 100 mM respectively. This plant at 100 mM salinity, compared to control, had less activity; that this decrease for GPX was significant but for APX was not. GPX enzyme was diminished at 100 mM salinity. Salicylic acid at 100 ppm, significantly encouraged the GPX and APX activity compared to control but at 150 ppm had opposite result and non-significantly decreased their activity. Interaction between salinity and salicylic acid for GPX and APX activity showed that these two enzymes at 50 mM salinity and 50 ppm salicylic acid, also at 50 mM salinity and 100 ppm salicylic acid had highest activity (Figures 2 and 3). Among different salinity levels, the highest GPX and APX activity was at 50 mM, then 25, 0 and 100 mM respectively. Myrtle at 100 mM salinity had less activity compared to control and this decrease for GPX was significant but for APX was not.

The highest level of SOD activity was seen at 50 mM salinity and then at 25, 0 and 100 mM respectively. SOD activity in control plants was higher than 100 mM and this difference was significant. Salicylic acid at level of 100 ppm showed highest level of SOD activity that it was significant. After that, the highest level of SOD activity was observed at 0, 50 and 150 ppm (Figure 4).

The highest level of prolin was observed at 50 mM salinity and then at 25, 0 and 100 mM. At 100 mM salinity, the prolin content was significantly reduced compared to control. The highest level of prolin content was seen at 50 ppm salicylic acid that this increase was significant. The least amount of prolin content was seen at 150 ppm and this decline was significant (Figure 5).

The highest level of glysin-betaein was observed at 25 mM salinity and then, at 0, 50 and 100 mM. In 100 mM salinity the amount of glysin-betaein was less than control plants and this slump was significant. With respect to the main effect of salicylic acid, at 50 ppm, glysin-betaein has highest level and this peak was significant; but difference between 0 and 100 ppm was not significant. The least amount of glysin-betaein was at 150 ppm that it was significant compared to other treatments except for control (Figure 6).

The highest quantity of ELI was at 100 mM salinity that it was noticeable compared to other treatments. Even though the least quantity of ELI was at 0 mM and its difference was significant compared to other treatments. Assessing the impact of salicylic acid showed that the least ELI was at 150 ppm that its difference between 0 ppm or 100 ppm was not significant (Figure 7).

The maximum amount of $H_2O_2$ was at 100 mM salinity and it was significantly different to other treatments. Among other different salinity treatments there was no significant difference. The main effect of salicylic acid on $H_2O_2$ showed that 150 ppm of salicylic acid cause to highest synthesis of $H_2O_2$ and its difference from other treatments was significant; then had a dwindle trend at 100, 50 and 0 ppm, respectively (Figure 8).

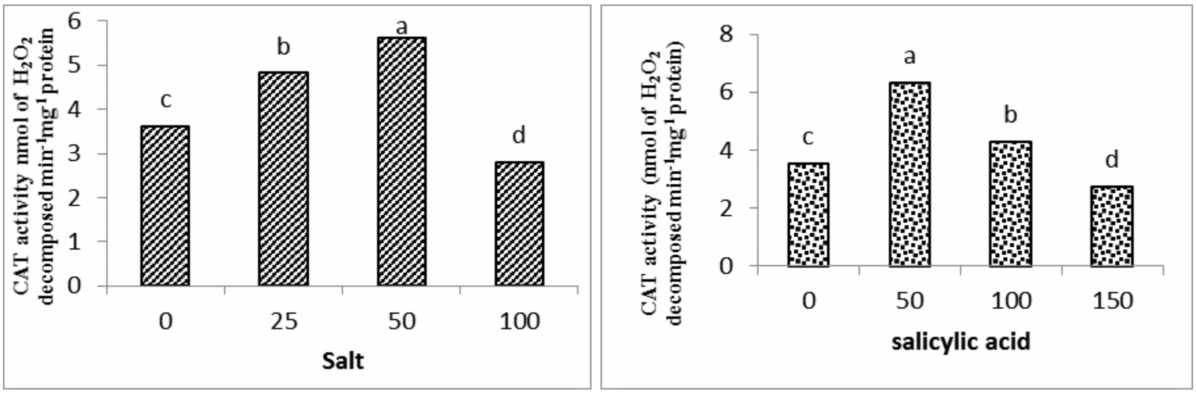

Figure 1. Effect of salinity and salicylic acid treatments on CAT activity.

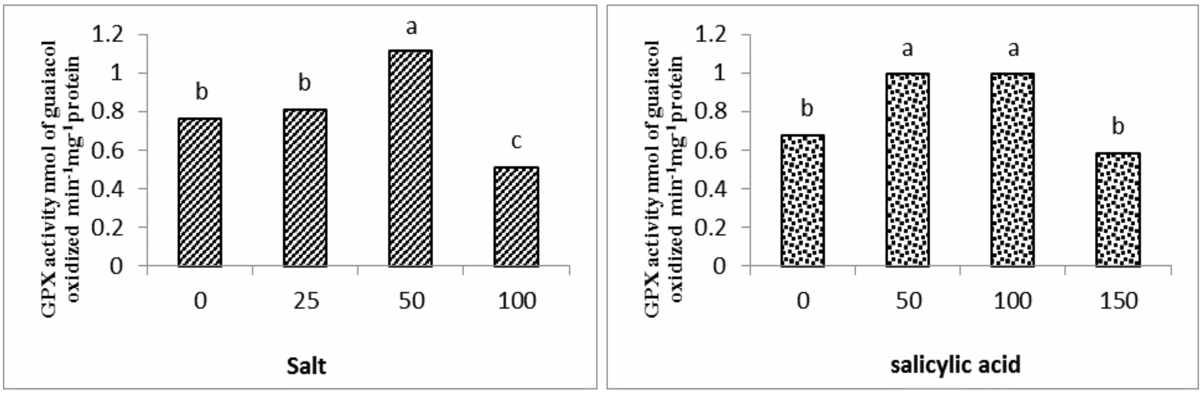

Figure 2. Effect of salinity and salicylic acid treatments on GPX activity.

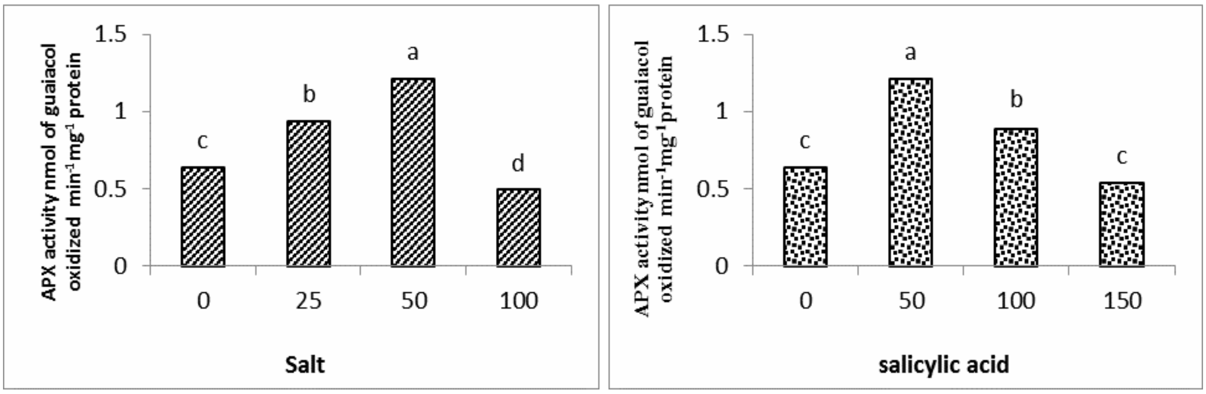

Figure 3. Effect of salinity and salicylic acid treatments on APX activity.

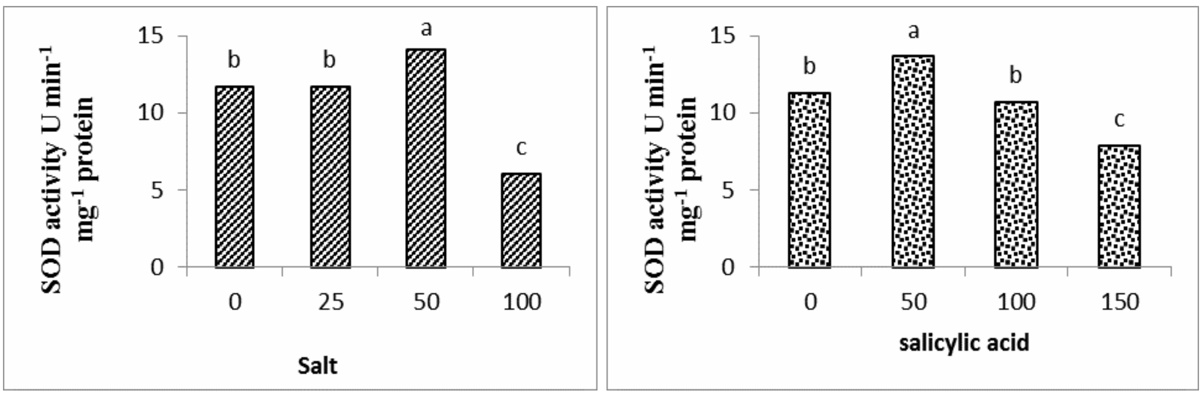

Figure 4. Effect of salinity and salicylic acid treatments on SOD activity.

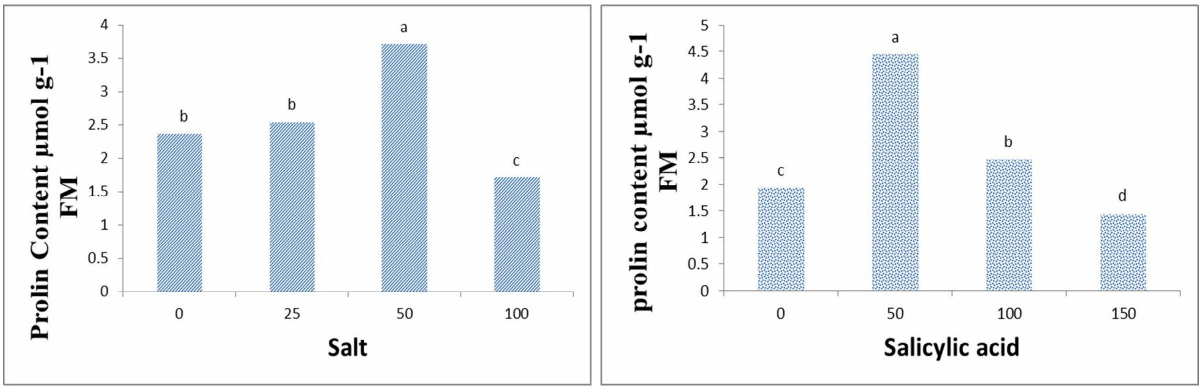


Figure 5. Effect of salinity and salicylic acid treatments on prolin content.

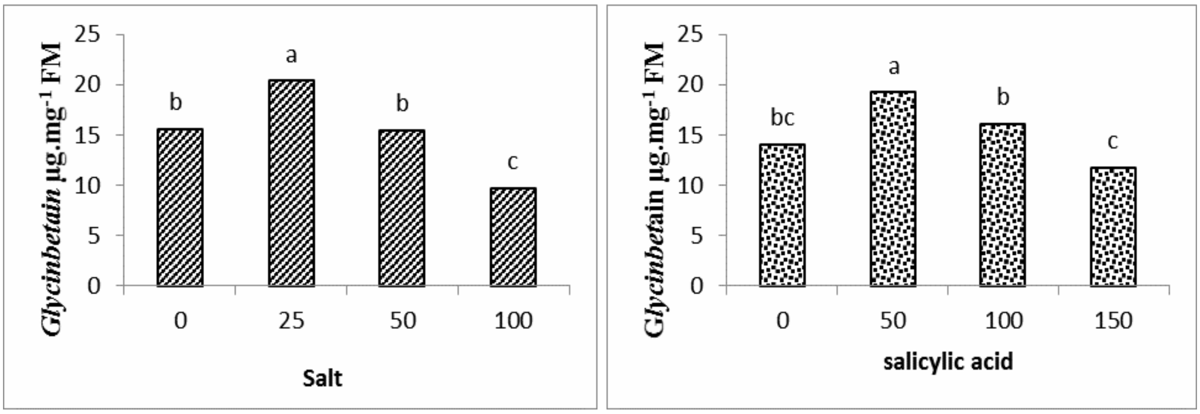


Figure 6. Effect of salinity and salicylic acid treatments on glycinebetaine activity.

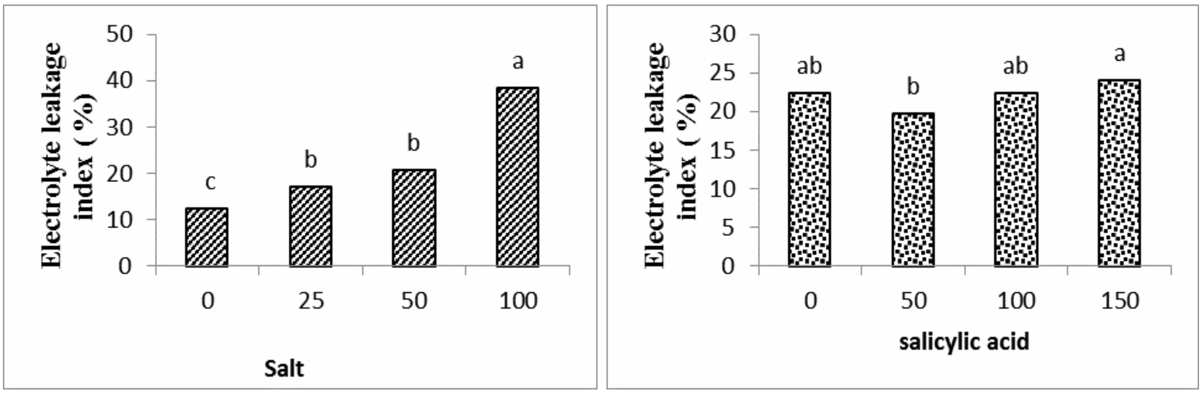


Figure 7. Effect of salinity and salicylic acid treatments on ionic leakage.

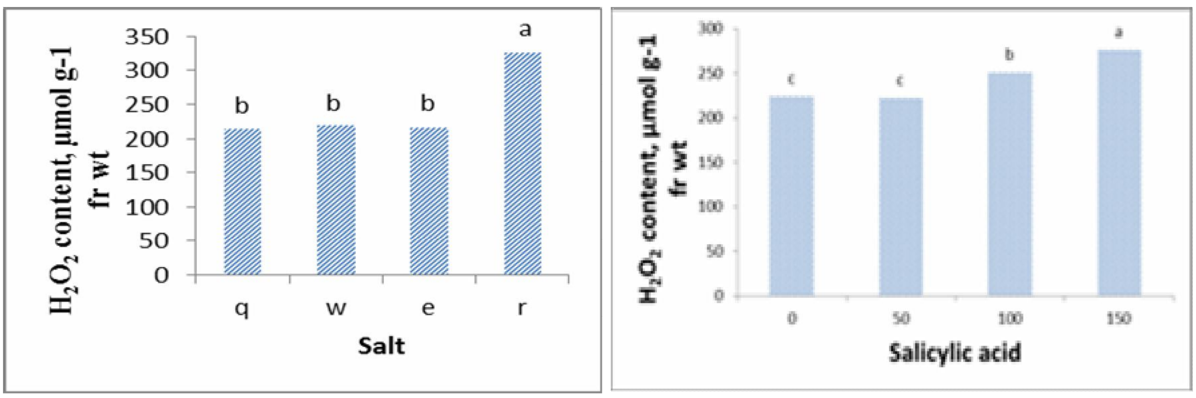


Figure 8. Effect of salinity and salicylic acid treatments on $H_2O_2$.

The decrease of CAT activity affected by 150 ppm salicylic acid was because of salinity tension conditions. Salicylic acid, inhibitor of CAT performance and by decrease of CAT activity and increase of $H_2O_2$ inside of plant (Horváth et al 2002). Also some reports acclaim that oxidative tensions deteriorate the CAT enzyme activity and escalate the amount of salicylic acid in rice, wheat, cucumber (Shim et al 2003) and in other plants (Sanchez-Casas & Klessig 1994). The improvement of effect between 50 ppm salicylic acid and 50 mM salinity, cause to increase antioxidant capacity of myrtle in direction of more production of CAT enzyme. Total consequence of salinity and salicylic acid impact and their interaction showed that 100 mM salinity and 150 ppm salicylic acid, reduce the CAT enzyme activity, hence plant toleraees this decline, because this reduction was not significant compared to control plants. But it shows a nosedive trend, that continues in higher concentrations of salinity and salicylic acid and at last, interrupts the physiologic status of plant. The highest activities of antioxidant system were obtained at 50 mM salinity and 50 ppm salicylic acid.

The least activity (significant) of GPX and APX was at 100 mM salinity and 150 ppm salicylic acid, that it can be representative of the injury to plant. The decrease of GPX enzyme at 100 mM salinity shows the ability of this level of salinity to reduce the antioxidant capacity. But GPX is not the main enzyme of $H_2O_2$ elimination and it has assistant contribution in $H_2O_2$ elimination, thus, this decrease of GPX cannot be seriously injurious to GPX. Leap of SOD activity cause to more production of $H_2O_2$ that it is poisonous to cell and should be immediately decomposed to water and oxygen by antioxidant defense system (Guo et al 2006). So, plant to get this purpose, utilizes other antioxidant enzymes to decompose the extra $H_2O_2$. Thus, it can be concluded that one of the reasons for increase of GPX activity in various levels of salinity tension is increase of SOD activity. Comparison of interaction between salinity and salicylic acid showed that addition of salicylic acid, improves the activity of peroxidase enzymes. It seems that higher concentrations of salicylic acid like 50 and 100 ppm besides of 50 mM salinity, cause to escalate the activity of GPX and APX enzymes that this result support research of Janda et al (1999). Eventually, results show that salicylic acid limitedly causes to remove of some disruptive impacts of salinity tensions especially prior to 50 mM.

Product of SOD activity ($H_2O_2$) is substrate of GPX and APX activity. So $H_2O_2$ can play as a signal to induce GPX and APX (Faize et al 2011). So, it is possible that a part of increase in GPX and APX activity, consequence of interaction between 50 mM salinity and 50 ppm salicylic acid also 50 mM salinity and 100 ppm salicylic acid, was due to rise of level of SOD enzyme. Decline of SOD at 100 mM salinity shows that this level of salinity could to reduce the antioxidant capacity of plant. The data of SOD confirms the data of CAT, GPX and APX, because interaction between 50 mM salinity and 50 ppm salicylic acid maximized the activity of each four antioxidant enzymes. Thus, it confirmed one of theories which acclaim that increase of CAT, GPX and APX enzymes is because of increase in SOD. $H_2O_2$ can play as signal for increasing of CAT, GPX and APX. At 50 mM salinity, by increasing the salicylic acid more than 50 ppm, the amount of SOD declined, especially there is the highest decrease of SOD at 100 mM salinity and 150 ppm salicylic acid and at this treatment the plant had injured, because the decline of antioxidant in treated plant was significant compared to control plant. These results showed that use of salicylic acid less than 50 ppm beside of 50 mM salinity, significantly increased the SOD activity compared to most of treatments. Salicylic acid improves the plant tolerance by increasing this enzyme and via this action, counteracts to tension. Impact of salicylic acid in maintenance of plant response in various oxidative tensions has been reported (Shirasu et al 1997).

Proline has different roles in osmotic tension included stability of proteins and cell membranes, sub-cell structures and protection of cell performances by remove the active oxygen elements (Chey & Li 2007). Results of this study showed a growth in prolin content in tensioned plants at 25 and 50 mM salinity; and supports the previous studies which acclaim this increase is due to defensive nature of prolin (El-Tayeb et al 2006). Research on black olives (Kouhifayegh et al 2013), rice (Weems et al 1999) and wheat (Wang et al 2007) under salinity tension, supports results of this study. Outcomes of this study are that apply the 50 mM salinity and 50 ppm salicylic acid cause to increase of prolin accumulation. It can be assumed that treatment with salicylic acid lead to the prolin accumulation (Ashraf & Foolad 2007) which is consistent with the results of other researchers (Misra & Saxena 2009).

Glycinebetaine appears in plant by tension crisis and has high correlations with plant growth in dry and salty environments (Hanson et al 1985). Khan et al (2000) reported that by leaping the salinity, concentration of glycinebetaine in *Atriplex* spp. has increased. According to the present study, by increase of salinity tension levels to 25 mM, plant to maintenance the status of cells and tissues, could to increase the glycinebetaine; then it was steady at 50 mM salinity, and at 100 mM salinity showed a significant drop compared to control plants. Results showed that myrtle at initial stages of tension, used glycinebetaine as a mechanism to counteract the tension but at higher levels of tension, this plant utilized other mechanisms to counteract the tension. The impact of salicylic acid on accumulation of glycinebetaine has been proved (Hanson et al 1985; Hussain et al 2011; Khan et al 2014). In the present study, at 50 ppm salicylic acid, prolin was climbed significantly compared to other compounds. Effect of salicylic acid showed that the highest ELI observed at 150 ppm that it was significant and confirms results of Bastam et al (2013). Comparison of the mean of squares showed that salinity tension cause to increase the electrolyte leakage. Assessing the interaction effects showed the highest amount of ELI was observed at 100 mM salinity and different levels of salicylic acid, which indicates that decrease of antioxidant enzymes is due to injury to cell such as plasma membrane. Present study appeals that exactly the highest level of salinity and salicylic acid was injurious to plant, but the plant tolerated this injury and it was compensable. Because when amount of ELI gets to 50, from this amount is not more tolerable for plants (Lim et al 1998). While, at investigated treatments, the amount of ELI was less than 50.

Hydrogen peroxide is one of the effective factors in composition of lipid peroxidation (Sairam et al 2005; Prasad et al 1994). Also, hydrogen peroxide can contribute production of OH free radicals (another factor of oxidative tension) (Willekens et al 1995). Results confirm that higher level of salinity tension cause to higher amount of hydrogen peroxide (Figure 8). Increase $H_2O_2$ under tension may be due to the conversion of oxygen to hydrogen peroxide by SOD activity (Parida & Das 2005). In 100 mM salinity ELI was climbed, especially at interaction treatments between 100 mM salinity and all salicylic acid treatments (Figure 8). Increase of $H_2O_2$ cause to improve the ELI and decline of catalase, so increase of ELI, was considerably because of ROS invasion to cell plasma membrane of myrtle. Myrtle can tolerate 100 mM salinity but it was disruptive and the plant was injured because during the increase of salinity at the same time $H_2O_2$ has been increased, it means defense system of the plant could not maintain the level of $H_2O_2$ at low rate efficiently.

**Conclusions**. This study shows that myrtle recognized the salinity tension and responses to that. Salinity tension under 50 mM could activate the defense system but injury indexes (ELI and $H_2O_2$) show that 100 mM salinity damage to plant partly, that probably is tolerance threshold for myrtle. Also, salicylic acid at 50 ppm had a positive influence on physiologic system but at 150 ppm had negative effect on antioxidant system. The best treatment combination to improve defense system was 50 mM salinity and 50 ppm salicylic acid.

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