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Protective Role of Salicylic Acid on *Rosmarinus officinalis L.* Plant in Response to Salinity

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ABSTRACT

This study was aiming to investigate the effect of different salinity levels, salicylic acid (SA) concentrations and their combination on plant growth, volatile oil production, physiological and biochemical characters of rosemary. Plants were treated with salinity levels at 0, 25, 50 and 100 mM NaCl and SA concentrations as 0, 0.2 and 0.4 mM. Plants responded to salinity via significant reduction in growth as well as relative water content (RWC) against control treatment. Salinity had no effect on volatile oil yield, even though the volatile oil % was increased. Also, salinity decreased total chlorophyll content and enhanced other Physiological and biochemical parameters as well as antioxidant enzyme (CAT, SOD and POX) activities comparative to unstressed plants. The % of N, P, and K were declined within salinity increase. In the meantime, Na and Cl were gradually increased in response to salinity level increase. SA foliar application alleviated the abovementioned salinity deleterious effects on growth, volatile oil production and the physiological and biochemical characters investigated.

Keywords: Rosemary, salinity, salicylic acid, antioxidant enzymes, proline, mineral content.

INTRODUCTION

Rosmarinus officinalis L. (Fam. Lamiaceae) native to the Mediterranean and Asia and had been used worldwide in folk medicine, as a spice and a natural preservative for its high antioxidant and antimicrobial Beninca *et al*., (2011). There is a great demand in industry on rosemary essential oil due its antibacterial, antioxidant and free radical scavenger properties which is related to phenolic diterpenes, carnosol. The essential oil main components 1,8-cineole (35.8%) is responsible for the anti-inflammatory, antiseptic, antispasmodic and anti-diabetic effect of the oil.

Limitation of water resources in different arid regions forced growers worldwide for extensively use water with higher salinity in the irrigation of different crops Gideon *et al*. (1999). Statistics indicate that 20% of world's farm land contain higher salt content and/or irrigated saline water Ghassemi *et al*., (1995). Salinity severely affect the medicinal crops production and the damage in some cases could reach more than 50% of the total yield Mahajan and Tuteja, (2005). Na^+ and Cl^- ions are mainly responsible for the adverse effect of saline water as they consider toxic to plants and causes deterioration in soil structure Hasegawa *et al*., (2000). For these reasons, salinity represent the main threat for locally and global sustainable food production Lobell *et al*., (2007).

For mitigation of antagonistic effects of salinity, various approaches was assumed to explore mechanisms for salinity tolerance. Plants tend to accumulate salicylic acid (SA, 2-hydroxy benzoic acid) as a responses under salinity stress Hela *et al*., (2009). Exogenous application of SA has been widely used to alleviate plant tolerance for salinity Jayakannan *et al*., (2015). SA organize K^+ leakage form root tissues and H^+-ATPase activity to help adverse the effect of high salinity Jayakannan *et al*., (2013) and this enhance Na^+/H^+ exchanger through plasma membrane to shrink Na^+ buildup in the cytosol. In addition, SA interfere with different physiological responses to augment abiotic stress condition i.e. osmotic adjustments, ion prohibiting, lipid oxidation reduction, kinases synthesis and oxidative system regulation.

Despite the facts of rosemary value and demand in international market, the growing criteria using water containing higher levels of salts is still lacking behind. Rosemary is believed to be relatively tolerant to salinity, partly by regulating stomatal opening and osmotic adjustment activation. Yet, the mechanism of salinity tolerant of rosemary regarding photo protection and antioxidant protection is still unknown Tounekti *et al*., (2011).

Thus, this study aimed to investigate the effect of salinity concentrations, SA levels and their interaction on the growth, and volatile productivity rosemary plant. In addition, to provide information for understanding the different mechanisms by which SA can protect the rosemary plants against salt stress.

MATERIALS AND METHODS

Two Pot experiment were carried out at the Experimental nursery of Horticulture Department, Faculty of Agriculture, Tanta University, Tanta, Egypt during 2014 and 2015 seasons for assessment the effects of salinity, salicylic acid (SA) and treatments interaction on growth, yield and some physiological and biochemical parameters of *Rosmarinus officinalis* L. plant.

Plant material: seedlings of rosemary were produced in the greenhouse in January and two months after, standardized seedlings were displaced into (30 cm) containers filled with previously desalted sand.

Salinity treatment: Plants was irrigated with saline water at 0, 25, 50 and 100 mM NaCl two weeks after transplanting. To prepare irrigation solution, calculated amount of NaCl was dissolved in water and concentrations adjustment was performed using portable EC meter.

Plants were irrigated with saline water every 7 days using 0.5 L irrigation water per pot. Irrigation started with 25mM saline water and was increased by 25mM every other irrigation time till reaching the targeted concentrations to avoid impulsive shock. Distilled water was used for control treatment using 0.5 L at the same period of salinity treatments.

Salicylic acid (SA) treatment: Dimethyl sulfoxide was used to dissolve salicylic acid (SA; 2-hydroxybenzoic acid) and later distilled water plus 0.02% Tween 20 was used to prepare irrigation water at 0, 0.2 and 0.4mM SA (pH6.5). The treatments were started one week after salinity treatment as foliar spray. SA was applied weekly in the early morning till the end of the experiment. The salinity and SA treatments arranged in factorial design

contained 12 treatments (4 x 3) with three replicates each. The experiment was repeated twice during 2014 and 2015 seasons and data was combined.

Growth parameters: Plant height (cm). Branch Numbers on the main stem was counted to obtain branch number. Sample of fresh weight of herb was measured (g) and then oven-dried at70°C for 48 h till constant weight to get the herb dry weight (g).

Relative water content (RWC %) was calculated according to Weatherley (1950).

Volatile oil % were determined according to British Pharmacopea (1963) hydro distillation method.

Total chlorophyll content was examined in leaves following Sadasivam and Manickam (1992).

Total soluble sugars (%) was analyzed in leaf samples following Dubois *et al*. (1956) method.

Proline content (µmol g-1 FW): (0.5g) of frozen leaf samples was used for free proline content (µmol g-1 FW) determination following Bates *et al*. (1973) procedures.

Antioxidant enzyme assays: following Bradford (1976) method, soluble protein contents of the enzyme extract were assayed. SOD (Ec 1. 15. 1.1) activity min-1 mg-1 protein was measured as described by Giannopolitis and Ries (1977). CAT (Ec 1. 11. 1.6) activity µmol min-1 mg-1 protein was spectrophotometrically estimated by Clairbone (1985) method. POX (Ec 1. 11. 1.7) activity µmol min-1 mg-1 protein was measured following to Shanon *et al*. (1966).

Nutrient elements: Nitrogen (%) was determined using modified micro Kjeldahal method according to Black *et al*. (1965). Phosphorus (%) was colorimetrically determined at 660 nm wave length according to Jackson (1978). Potassium (%) was estimated by using a flame photometer which was standardized with standard solution according to Chapman and Pratt (1961). Sodium and Chloride ($mgg^{-1}FW$) was colorimetrically measured using a spectrophotometer as described by Munns *et al*., (2010).

Statistical analysis: The data was statistically analyzed using MSTAT-C program, USA. Data was combined for two experiments ($n=6$). Means were compared using LSD test at 0.05 level.

RESULTS AND DISCUSSION

Effect of salinity, SA and their interaction on plant height (cm) and branch number.

The plant height as well as branch numbers was significantly and gradually reduced with increasing salinity level compared with the control to reach the highest values at 100 mM treatment as results in Table (1) indicates. However, applying SA significantly enhanced the plant height and branch number comparative to the control. Moreover, when salinity was combined with SA, the plant height and branch numbers was reduced at all salinity treatment particularly at 0.4 mM SA.

Table 1. Growth parameters of *Rosmarinus officinalis* L plant as affected by salinity, salicylic acid (SA) treatments and their interaction.

Salinity (mM)	SA (mM)	Plant height (cm)	Number of branches per plant	Relative water content (RWC %)	Herb fresh weight (g)	Herb dry weight (g)
0		30.67 ± 1.03	7.56 ± 0.58	87.56 ± 0.77	55.67 ± 1.71	10.81 ± 0.08
25		28.11 ± 1.03	7.22 ± 0.72	86.67 ± 0.58	53.78 ± 1.40	10.28 ± 0.15
50		25.22 ± 1.40	6.56 ± 0.58	83.67 ± 0.77	50.78 ± 1.53	9.87 ± 0.15
100		22.22 ± 1.51	5.44 ± 0.58	79.33 ± 1.03	45.22 ± 0.89	8.96 ± 0.31
LSD at P ≤ 0.05 %		1.76	0.54	0.92	1.36	0.13
	0	23.25 ± 2.98	5.75 ± 1.87	81.75 ± 3.26	46.50 ± 5.28	9.24 ± 0.41
	0.2	26.25 ± 3.49	6.58 ± 2.29	84.75 ± 1.87	51.58 ± 4.96	10.07 ± 0.46
	0.4	30.17 ± 5.08	7.75 ± 1.87	86.42 ± 1.98	56.00 ± 4.35	10.63 ± 0.47
LSD at P ≤ 0.05 %		0.92	0.60	0.72	0.99	0.20
	0	27.67 ± 0.58	6.67 ± 0.58	86.67 ± 1.15	50.67 ± 2 .08	10.39 ± 0.04
0	0.2	31.00 ± 1.00	7.33 ± 0.58	87.33 ± 0.58	56.67 ± 1.53	10.77 ± 0.10
	0.4	33.33 ± 1.53	8.67 ± 0.58	88.67 ± 0.58	59.67 ± 1.53	11.26 ± 0.10
	0	25.00 ± 1.00	6.33 ± 0.58	85.67 ± 0.58	49.33 ± 1.53	9.40 ± 0.16F
25	0.2	27.67 ± 0.58	7.00 ± 1.00	86.67 ± 0.58	53.67 ± 1.53	10.40 ± 0.14
	0.4	31.67 ± 1.53	8.33 ± 0.58	87.67 ± 0.58	58.33 ± 1.15	11.04 ± 0.15
	0	21.33 ± 1.15	5.67 ± 0.58	80.33 ± 1.15	45.67 ± 1.53	9.073 ± 0.12
50	0.2	24.67 ± 1.53	6.33 ± 0.58	84.33 ± 0.58	50.33 ± 1.53	10.05 ± 0.12
	0.4	29.67 ± 1.53	7.67 ± 0.58	86.33 ± 0.58	56.33 ± 1.53	10.49 ± 0.20
	0	19.00 ± 1.00	4.33 ± 0.58	74.33 ± 1.53	40.33 ± 0.58	8.093 ± 0.39
100	0.2	21.67 ± 1.53	5.67 ± 0.58	80.67 ± 0.58	45.67 ± 1.53	9.037 ± 0.44
	0.4	26.00 ± 2.00	5.67 ± 0.58	83.00 ± 1.00	49.67 ± 0.58	9.743 ± 0.11
LSD at P ≤ 0.05 %		1.84	1.20	1.44	2.00	0.41

Values are means ± SD. Means in the same column for each trait are the average of two independent experiments (n = 6). Values significantly differ for each other according to LSD test at 5 % (P ≤ 0.05).

The reduction occurred in plant height and branching after salinity possibly resulted from either cell division or cell enlargement reduction (Yasseen *et al*., 1987). In the meantime, Munns (2002) revealed that salinity stress decrease the capability of plants to water absorbance, leading to retarded growth combined with various physiological responses corresponding to the case of drought. Moreover, plant develop general growth retard to adapt to salt salinity (Qaderi *et al*., 2006). Also, these results could be a logical results for the accumulation of toxic ions Na^+ and Cl^- and/or the osmatic imbalance caused to plant and by salt accumulation (Hajiboland *et al*., 2010).

Effect of salinity, SA and their interaction on relative water content (RWC %).

RWC was sharply decrease especially at the highest salinity level (100 mM) was applied. In contrast, applying SA whether at (0.2 or 0.4 mM) significantly increased RWC comparative to untreated plants Table (1). However, deleterious effect of salinity on RWC was alleviated when

SA was used under any salinity level. Reducing leaves RWC is a reasonable result explains the growth inhibition as it reflect the plant water status. Whereas, SA foliar application decrease leaf transpiration to increase leaf diffusive resistant which finally increases RWC (Karlidag *et al*., 2009) or reacting to stress through compatible osmolytes building up in plants tissues (Kabiri *et al*., 2014). Hassan and Ali (2014) previously found supportive results by on gladiolus.

Effect of salinity, SA and their interaction on herb fresh and dry weights (g).

Raising salinity levels up to 100 Mm gradually reduced plant fresh weight and the differences were significant relatively to the control Table (1). Meanwhile, both SA applications significantly enhanced the fresh weight per plant relative to untreated plants and the treatment of 0.4mM was more effective in this regard than 0.2 mM Table (1). Moreover, the combination between salinity and SA levels minimized the drop of fresh weight resulted by salinity. The same pattern was detected in case of dry weight. Leveling up salinity gradually and significantly reduced dry weight compared with the control. Instead, SA significantly increased the dry weight relative to the control. Under any salt level, SA increased the dry weight of rosemary comparing with salt treated plants.

The protective role of SA in leveling up plant tolerance via membrane protection resulted in alleviated dry matter accumulation in response to salinity stress Misra and Saxena (2009). Under stress condition SA works as signal molecule responsible for moderating stressed plant responses in order to mitigating plant injurious under different environmental stresses Hela *et al*., (2009). Other investigators Hassan *et al*. (2013), Abd El-Mageed *et al*. (2016) results previously confirmed these finding.

Effect of salinity, SA and their interaction on volatile oil % and yield (ml/plant).

Regarding volatile oil %, the highest significant percentage was obtained by applying salinity at 100 mM comparative to the control, also the oil % was promoted after SA treatments Table (2). The highest SA dose (0.4 mM) gave the highest volatile oil percentage (1.04 %) as compared with control (0.91%). The percentage of volatile oil increased when salinity was combined with SA. The highest volatile oil percentage (1.32%) was recorded when plants sprayed with SA at 0.2mM under 100 mM NaCl treatment. In the meantime, volatile oil yield/plant slightly improved due to salinity without significant difference between any salinity treatment and control in this respect. However, oil yield was increased relatively to the control when SA treatment was applied.

Table 2. Physiological and biochemical parameters of *Rosmarinus officinalis* L plant as affected by salinity, salicylic acid (SA) treatments and their interaction.

Salinity (mM)	SA (mM)	Volatile oil %	Volatile oil yield (ml/plant)	Chlorophyll content (mg g^{-1} FW)	Total soluble sugars (%)	Proline content µmol g^{-1} FW
0		0.89 ± 0.02	0.50 ± 0.02	1.22 ± 0.02	7.96 ± 0.09	1.61 ± 0.03
25		0.95 ± 0.02	0.51 ± 0.02	1.18 ± 0.01	8.37 ± 0.08	1.93 ± 0.04
50		1.00 ± 0.02	0.51 ± 0.02	1.13 ± 0.03	9.11 ± 0.04	2.06 ± 0.02
100		1.15 ± 0.16	0.52 ± 0.06	0.89 ± 0.02	10.02 ± 0.07	2.13 ± 0.02
LSD at P ≤ 0.05 %		0.14	0.06	0.04	0.07	0.04
	0	0.91 ± 0.05	0.32 ± 0.04	1.01 ± 0.07	8.20 ± 0.22	1.87 ± 0.15
	0.2	1.04 ± 0.15	0.39 ± 0.09	1.89 ± 0.06	8.96 ± 0.21	1.94 ± 0.05
	0.4	1.04 ± 0.06	0.44 ± 0.07	2.16 ± 0.05	9.44 ± 0.21	1.98 ± 0.08
LSD at P ≤ 0.05 %		0.11	0.05	0.009	0.05	0.03
	0	0.84 ± 0.02	0.43 ± 0.02	1.157 ± 0.03	7.56 ± 0.10	1.57 ± 0.06
0	0.2	0.87 ± 0.01	0.49 ± 0.01	1.217 ± 0.01	7.97 ± 0.05	1.63 ± 0.02
	0.4	0.96 ± 0.03	0.57 ± 0.02	1.277 ± 0.01	8.34 ± 0.12	1.64 ± 0.02
	0	0.88 ± 0.01	0.43 ± 0.01	1.120 ± 0.01	7.81 ± 0.09	1.87 ± 0.06
25	0.2	0.95 ± 0.02	0.51 ± 0.02	1.177 ± 0.02	8.53 ± 0.10	1.94 ± 0.02
	0.4	1.01 ± 0.03	0.59 ± 0.03	1.237 ± 0.02	8.78 ± 0.05	1.99 ± 0.04
	0	0.94 ± 0.02	0.43 ± 0.01	0.990 ± 0.03	8.02 ± 0.03	1.99 ± 0.03
50	0.2	1.01 ± 0.03	0.51 ± 0.02	1.047 ± 0.02	9.42 ± 0.06	2.07 ± 0.02
	0.4	1.05 ± 0.01	0.59 ± 0.02	1.133 ± 0.02	9.89 ± 0.03	2.12 ± 0.03
	0	0.99 ± 0.03	0.40 ± 0.01	0.757 ± 0.03	9.43 ± 0.06	2.06 ± 0.03
100	0.2	1.32 ± 0.42	0.60 ± 0.17	0.913 ± 0.02	9.91 ± 0.03	2.13 ± 0.02
	0.4	1.13 ± 0.02	0.56 ± 0.02	0.993 ± 0.03	10.73 ± 0.12	2.19 ± 0.02
LSD at P ≤ 0.05 %		0.22	0.09	0.017	0.11	0.05

Values are means ± SD. Means in the same column for each trait are the average of two independent experiments (n = 6). Values significantly differ for each other according to LSD test at 5 % (P ≤ 0.05).

Since volatile oil is consider as a secondary metabolite compound it could be assumed that salinity increase oil % increasing in conjunction with increasing TSS as data indicates. Meanwhile, SA treatment increased the volatile oil percentage and yield of rosemary plant. This may be related to the increase in pant growth occurred by SA, population changes of oil gland in leaf, and the advantageous role of SA on mono or sesqueterpene biosynthesis Rowshan *et al*., (2010). These results support the findings of Kazemi and Shirzadeh (2012) who found that the treatment of SA was significantly improved the volatile oil of rosemary.

Effect of salinity, SA and their interaction on total chlorophyll content (mg g^{-1} FW).

Salinity treatments significantly reduced total chlorophyll content compared to control Table (2). On the other hand, SA treatment significantly and gradually enhanced total chlorophyll content at any SA concentrations. Regarding the interaction between salinity and SA treatments, the adversative effects of salinity was ameliorated due to any SA dose however 0.4 mM treatment was superior to 0.2 mM in this respect.

One the known effect of salinity is the deterioration of chlorophyll content. Under salinity, chloroplast structure as well as the photosynthetic apparatus are subjected to degradation including photo oxidation, biosynthesis inhibition, and increasing chlorophyll enzymatic degradation Kabiri *et al*., (2014). In this regard, Kiarostami *et al*., (2010) found a progressive decrease in chlorophyll under salinity in rosemary plant and the effect increase with alleviating salinity concentration. However, SA treatment positively affected the total chlorophyll content and ameliorated the adverse effects of salinity. In this regard, Singh and Usha (2003) found that chlorophyll content increased significantly with SA application under stress as compared to the stressed plants without SA.

Effect of salinity, SA and their interaction on total soluble sugars (%).

TSS gradually increased under salinity level and was maximized by applying the highest salinity level Table (2). Also, TSS % was increased along with increasing SA level particularly at 0.4 mM treatment. Rosemary plants grown under salinity and sprayed with SA recorded higher TSS values than the individual treatment of NaCl or SA. Increasing TSS may occurred as response to increase cells osmotic pressure under salt stress Teixeira and Pereira (2007) and/or to support metabolism, and provide energy supply for stress relieve Slama *et al*., (2007). Additionally, increasing sugar levels and subsequent tissues osmosis gradients after SA treatment is predicted to preserve water loss, chloroplasts protection and accelerate plant growth under stress conditions Amin *et al*., (2009).

Effect of salinity, SA and their interaction on proline content (µmol g^{-1} FW).

Proline content of rosemary plant was maximized by 100 mM NaCl treatment Table (2). Also, both SA levels significantly increased proline content compared with untreated plants. Moreover, the interacted treatments increased the proline content in rosemary leaves compared with the individual application with salinity or SA treatment. The treatment of 100 mM NaCl combined with 0.2 or 0.4 mM SA recorded the highest proline content in rosemary leaves. proline content substantial increase after SA and NaCl treatments, may be considered one of many adaptation strategies by plants to survive under stress which is documented in case of proline accumulation under salt stress (Misra, and Gupta, 2005). Also, Silva-Ortega *et al*. (2008) explained that Proline adjust intracellular osmatic and protect photosynthetic activity as it accumulate under salt stress Siddiqui *et al*., (2010). Treatments interaction increased proline content and antioxidant enzymes and hence the stress generated by NaCl was alleviated Yusuf *et al*., (2008).

Effect of salinity, SA and their interaction on antioxidant enzyme activity

The response of CAT, SOD and POX enzyme activities resulted for applying salinity or SA was tabulated in Table (3). Data showed that salinity treatment positively affected enzyme activities since all levels of NaCl significantly increased CAT, SOD and POX enzyme activities comparative to untreated plants. A gradual increase was detected in the activity of enzymes with increasing salinity dose and the highest activities in CAT, SOD and POX were obtained by the treatment of 100 mM NaCl.

The same direction was observed when SA treatments were applied. Both SA levels significantly increased CAT, SOD and POX enzyme activities in rosemary leaves compared with untreated plants. Applying SA at 0.4 mM was more effective than the treatment of 0.2 mM in this concern Table (3). When SA treatments were combined with salinity CAT, SOD and POX enzymes activities increased as well than those obtained by SA treatment alone. The highest values in this regard was obtained by the treatment of 100 mM NaCl combined with 0.4 mM SA.

Table 3. Antioxidant enzyme activity of *Rosmarinus officinalis* L plant as affected by salinity, salicylic acid (SA) treatments and their interaction.

Salinity (mM)	SA (mM)	CAT µmol min-1 mg-1 protein	SOD units min-1 mg-1 protein	POX µmol min-1 mg-1 protein
0		1.13 ± 0.02	0.84 ± 0.19	17.65 ± 0.11
25		1.20 ± 0.01	1.34 ± 0.74	19.48 ± 0.05
50		1.54 ± 0.02	1.02 ± 0.18	22.00 ± 0.06
100		1.75 ± 0.02	0.98 ± 0.14	25.94 ± 0.18
LSD at P ≤ 0.05 %		0.011	0.10	0.20
	0	1.30 ± 0.04	1.22 ± 0.68	18.55 ± 0.15
	0.2	1.41 ± 0.06	0.87 ± 0.13	21.73 ± 0.37
	0.4	1.50 ± 0.04	1.06 ± 0.18	23.52 ± 0.26
LSD at P ≤ 0.05 %		0.008	0.11	0.08
	0	1.06 ± 0.02	0.71 ± 0.08	16.53 ± 0.05
0	0.2	1.13 ± 0.02	0.75 ± 0.14	17.63 ± 0.18
	0.4	1.21 ± 0.01	1.06 ± 0.13	18.77 ± 0.12
	0	1.14 ± 0.01	2.33 ± 0.11	17.93 ± 0.03
25	0.2	1.21 ± 0.02	0.80 ± 0.05	19.30 ± 0.07
	0.4	1.25 ± 0.01	0.89 ± 0.06	21.22 ± 0.04
	0	1.43 ± 0.01	0.95 ± 0.06	19.48 ± 0.06
50	0.2	1.52 ± 0.02	0.93 ± 0.13	22.18 ± 0.03
	0.4	1.67 ± 0.02	1.18 ± 0.21	24.34 ± 0.08
	0	1.56 ± 0.02	0.88 ± 0.06	20.25 ± 0.08
100	0.2	1.77 ± 0.02	0.99 ± 0.05	27.79 ± 0.37
	0.4	1.86 ± 0.02	1.09 ± 0.21	29.76 ± 0.10
LSD at P ≤ 0.05 %		0.017	0.21	0.15

Values are means ± SD. Means in the same column for each trait are the average of two independent experiments (n=6). Values significantly differ for each other according to LSD test at 5 % (P ≤ 0.05).

It is evident that salinity trigger oxidative stress Malik *et al*., (2011) as a result of generating reactive oxygen species (ROS). The presence of ROS prompt plant cells antioxidant enzymatic system for ROS scavenger. Superoxide dismutation causes cell membrane damage and SOD is the responsible enzyme for catalyzing superoxide into hydrogen peroxide and oxygen. In the same respect the toxic damage of hydrogen peroxide is being divided to oxygen and water by CAT and/or POX Sairam *et al*., (2005). Reports indicate that POX activity is related to plant growth retard resulted from salt stress conditions which consider important defense mechanism Agarwal and Pandey, (2004).

Rosemary plants sprayed with SA in current study showed higher activity of antioxidant enzymes. It is well documented that, SA is a salinity tolerance response alleviate antioxidant machinery Fatma *et al*., (2013). Additionally, SA exogenous application was found to enhance CAT, POX and SOD activities under stress environments (Yusuf *et al*., 2008; Hassan and Ali, 2014).

Effect of salinity, SA and their interaction on nutrient elements content

As indicated in Tables (4) salinity treatments affected nutrient elements contents in rosemary leaves. Increasing salinity level gradually decreased N, P and K % to reach the lowest values at the highest salinity level. Meanwhile, salinity treatment significantly increased Na and Cl contents in rosemary leaves relative to untreated plants. The highest contents of Na and Cl were recorded by applying the treatment of NaCl at 100 mM.

Table 4. Mineral content of *Rosmarinus officinalis* L plant as affected by salinity, salicylic acid (SA) treatments and their interaction.

Salinity (mM)	SA (mM)	N (%)	P (%)	K (%)	Na ($mgg^{-1}FW$)	Cl ($mgg^{-1}FW$)
0		1.91 ± 0.02	0.42 ± 0.01	2.15 ± 0.02	2.85 ± 0.07	4.13 ± 0.06
25		1.83 ± 0.01	0.40 ± 0.01	1.93 ± 0.01	3.11 ± 0.05	5.33 ± 0.09
50		1.75 ± 0.02	0.36 ± 0.01	1.90 ± 0.02	3.62 ± 0.06	6.27 ± 0.11
100		1.69 ± 0.01	0.34 ± 0.01	1.80 ± 0.02	4.46 ± 0.09	7.72 ± 0.14
LSD at P ≤ 0.05 %		0.04	0.01	0.01	0.08	0.17
	0	1.72 ± 0.06	0.35 ± 0.04	1.90 ± 0.07	4.79 ± 0.28	7.84 ± 0.37
	0.2	1.82 ± 0.05	0.38 ± 0.03	1.95 ± 0.04	3.02 ± 0.21	5.15 ± 0.30
	0.4	1.85 ± 0.04	0.41 ± 0.03	1.98 ± 0.04	2.72 ± 0.11	4.60 ± 0.17
LSD at P ≤ 0.05 %		0.01	0.01	0.02	0.06	0.08
	0	1.86 ± 0.03	0.39 ± 0.02	2.11 ± 0.03	3.09 ± 0.11	4.25 ± 0.08
0	0.2	1.92 ± 0.02	0.42 ± 0.01	2.16 ± 0.02	2.88 ± 0.09	4.12 ± 0.07
	0.4	1.97 ± 0.02	0.45 ± 0.02	2.19 ± 0.01	2.58 ± 0.02	4.03 ± 0.02
	0	1.77 ± 0.02	0.36 ± 0.01	1.90 ± 0.02	3.65 ± 0.06	6.99 ± 0.15
25	0.2	1.84 ± 0.02	0.41 ± 0.01	1.94 ± 0.01	3.00 ± 0.05	4.84 ± 0.08
	0.4	1.89 ± 0.01	0.43 ± 0.01	1.96 ± 0.02	2.67 ± 0.03	4.17 ± 0.05
	0	1.67 ± 0.02	0.33 ± 0.02	1.84 ± 0.03	4.96 ± 0.08	9.08 ± 0.12
50	0.2	1.77 ± 0.02	0.37 ± 0.01	1.90 ± 0.02	3.08 ± 0.05	5.28 ± 0.13
	0.4	1.81 ± 0.02	0.39 ± 0.01	1.94 ± 0.02	2.83 ± 0.06	4.47 ± 0.08
	0	1.59 ± 0.02	0.31 ± 0.01	1.76 ± 0.03	7.46 ± 0.15	11.05 ± 0.15
100	0.2	1.76 ± 0.01	0.34 ± 0.02	1.81 ±0.01	3.13 ± 0.09	6.38 ± 0.14
	0.4	1.71 ± 0.02	0.37 ± 0.02	1.84 ± 0.01	2.78 ± 0.02	5.74 ± 0.12
LSD at P ≤ 0.05 %		0.02	0.02	0.02	0.12	0.15

Values are means ± SD. Means in the same column for each trait are the average of two independent experiments (n = 6). Values significantly differ for each other according to LSD test at 5 % (P ≤ 0.05).

Oppositely, SA treatment resulted in significant increase in N, P and K % comparative to the control and the highest % were obtained with higher SA level (0.4 mM). Moreover, SA treatment markedly decreased Na and Cl contents in rosemary leaves compared with the control and the reduction was gradually within SA level Tables (4). Both SA treatments improved the % of N, P, and K and Mg under any salinity level compared with SA-untreated plants. In addition, SA treatment significantly reduced Na and Cl contents in salt treated plants.

N% reduction in plant tissues under salinity condition is a direct result of accumulation of toxic ions Na^+ and Cl^-. Sodium accretion in plants causes cell membrane depolarization Suhayda *et al*., (1990) and this cause a competition between NO_3^-/Cl^- at the sites for ion transport Cram, (1983). Furthermore, the presence of Na^+ causes H_2PO_4 precipitation with Ca^{2+} ions leading to drop in P uptake Marschner, (1995). Also the competition between Na^+ and K^+ causes reduction of internal K^+ at higher external NaCl Botella *et al*., (1997). Increasing salt concentration adversely affect ion equilibrium in plant since salinity causes a significant decrease in some important nutrients and consequently ion toxicity, osmotic stress and nutrient deficiency were occurred Sairam and Tyagi (2004).

SA treatment also stimulated the uptake of N, P and K by rosemary plants even under salt stress. Otherwise, Na and Cl reduced significantly due to SA application. The effect of SA on mineral uptake could reverse the harmful effects of salinity. Therefore, mineral accumulation adjustment by SA applications is a possible defense system for alleviation salt stress Karlidag *et al*., (2009).

In this respect, Hela *et al*., (2009) and Nazar *et al*., (2011) suggested the role of exogenous application of SA as a plant growth regulator to mend up plant resistance to salinity stress since SA treatment inhibited Na+ and Cl- accumulation, but stimulated K+, Ca+2 and Mg+2 contents of stressed plants. The defensive role of SA in regulation of ion uptake has also been reported (Guo *et al*., 2013; Fayez and Bazaid, 2014).

CONCLUSION

It could be concluded that the foliar application of SA alleviated the negative effects of salinity on growth, herb and volatile oil yields and the physiological and biochemical characters investigated. Moreover, the increment of antioxidant enzyme activities and accumulation of proline as a result of SA treatment are suggested to involve as part of the defense against salinity in rosemary plant. To alleviate the negative effects of salinity on rosemary plant, treatment of SA at 0.4 mM was recommended.

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التأثير الوقائى للاجهاد الملحي باستخدام حمض الساليسيليك علي نبات حصالبان

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أجريت هذه التجربة لدراسة تأثير معاملات الملوحة، وحمض الساليسيليك وكذلك التداخل بينهما علي النمو والمحصول ومحتوي الزيت الطيار وكذلك بعض المقاييس الفسيولوجية والبيوكيماوية ومحتوى العناصر في الاوراق لنبات حصالبان. التركيزات المستخدمة من كلوريد الصوديوم كمصدر للملوحة كانت صفر، ٢٥، ٥٠ و ١٠٠ مللي مولر بينما التركيزات المستخدمة من حمض الساليسيليك كانت صفر، ٠.٢ و ٠.٤ مللي مولر. وأدت معاملات الملوحة الي انخفاض معنوي في ارتفاع النبات، عدد الفروع، الوزن الطازج والجاف، محتوي الماء النسبي بالأوراق مقارنة بنباتات الكنترول. وبالرغم من ازدياد نسبة الزيت الطيار نتيجة المعاملة بالملوحة الا أن محصول الزيت لم يتغير معنويا مقارنة بالكنترول. كما أدت الملوحة الي نقص معنوي في محتوي الأوراق من الكلوروفيل بينما ازدادت السكريات الذائبة، البرولين و نشاط الانزيمات المضادة للأكسدة مقارنة بالنباتات الغير معاملة. بالاضافة الي ذلك انخفض محتوى الاوراق من عناصر النيتروجين، والفوسفور والبوتاسيوم بزياده تركيز الملوحة بينما إزداد تركيز كل من الصوديوم والكلوربالأوراق. وعلي العكس من ذلك فقد أدت المعاملة بحمض الساليسيليك الى التغلب على التأثيرات الضارة للملوحة على كل المقاييس الخضرية والفسيولوجية والبيوكيماوية السابق ذكرها.

كلمات البحث: حصالبان -الملوحة –حمض الساليسيليك - مضادات الأكسدة – البرولين – المحتوي المعدني.