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Research Article

Interaction of Salicylic Acid and Salinity on Essential Oil, Polyphenolic Content, Antioxidative Capacity and physiological Characteristics of *Matricariachamomile*

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ABSTRACT

Medicinal and aromatic plants are increasingly used in several fields such as agroalimentary, perfumes, pharmaceutical industry and natural cosmetic products. In the past few years, interest in the cultivation of medicinal plants under different environmental conditions has increased, because plant antioxidants and essential oils which are involved in preservation of human health can be induced under environmental stresses. *Matricariachammomile* are medicinal and aromatic plants belonging to the Asteraceae family, which have many pharmaceutical properties. In the present study, seeds of *M.chammomile* were grow in the greenhouse with the mean day/night temperature and relative humidity of 29 ± 4˚C, 38 ± 5% RH/17 ± 2˚C, 59 ± 5% RH respectively. To investigate the effects of Interaction of salicylic acid (SA) and salinity (Na) on secondary metabolite andphysiological characteristics of *M. chammomile*., Fourteen weeks old plants were subjected to different levels of salinity [control, 50, 100, and 150 mg l^{-1}] and salicylic acid [control, 150, 300, and 450 mg l^{-1}]. Our results showed that Interaction of SA and Na caused a significant increase in prolinecontent, photosynthetic pigments and antioxidant activity in*M. chammomile* but phenolic compounds have shown different profiles. polyphenol components were identified and analyzed by HPLC method. The main components of polyphenols were chloregeni acid, caffeic acid, catechin, sinapic acid, hesperidin, ellagic acid, quercetin andeugenol. The proportions of these main compounds were induced by moderate salinity and Interaction of salicylic acid and salinity.

Key word: Chamomile, salicylic acid, Salt stress,polyphenol components, antioxidant activity

INTRODUCTION

Fifteen percent of total agricultural lands of Iran have salt in water or soil. Salt stress can affect several physiological processes, from seed germination to plant development. The complexity of the plant response to salt stress can be partially explained by the fact that salinity imposes both an ionic and an osmotic stress (Pasternak, 1987). In such conditions cultivation of resistant plants is one way to utilize these lands and therefore the selection of suitable crops, which could cope with these conditions, is a necessity (Mirmohamadimeibodi

and Gharaiazi, 2002). The outcome of these effects may cause membrane damage, nutrient imbalance, altered levels of growth regulators, enzymatic inhibition and metabolic dysfunction, including photosynthesis which ultimately leads to plant death (Mahajan and Tuteja, 2005). On the other hand, salinity may increase the secondary products of plant (De-Abreu and Mazzafera, 2005. Hernaendez, et al. 2006)

*M. Chamomilla*is a species of aromatic annual herbs belonging to the family of Asteraceae (Salamon, 1992). Chamomile is a medicinal plant that is native to Europe (Pourohit and Vyas, 2004) and northern and western Asia, has been naturalized in Australia and North America, and is extensively cultivated in Hungary, Romania, Bulgaria, the former Yugoslavia, Germany, Greece, Argentina and Egypt (Nidagundi and Hegde, 2006). Chamomile is preferred for its pleasant taste and calming, sedative effects, as well as its long established medicinal properties. (Petronilho et al., 2012). Additional beneficial properties, such as anti-inflammatory, anti-spasmodic, anti-allergic and anti-bacterial, have been attributed to chamomile (Buono-Core et al., 2011).*M. chamomilla*has revealed the presence of flavonoids, sesquiterpenes, and coumarins (Ramadan et al., 2006).

Salicylic acid (SA) is a water-soluble antioxidant compound, which is found in the leaves and generative plant parts, helps the plants to resist against unfavorable environmental conditions. However, sometimes, it causes pest and disease tolerance through increasing aromatic substances (B.Arberg. 1981). It has been demonstrated that SA significantly increases EO content in some aromatic plants (Rowshan et al., 2013; Saharkhiz and. Goudarzi, 2014)application of exogenous salicylic acid improves essential oil content in chamomile cultivars under environmental heat stress conditions (Ghasemi et al., 2016). Total soluble phenols increased *M. chamomilla* after NaCl and SA treatments, but root lignin content was not affected (Kovacik et al., 2009b). Exogenous SA or methyl salicylate could induce the expression of many defense genes (Wen et al. 2005). A significant increase in the synthesis of flavonoids in response to application of SA was observed in various medicinal plant species like *M.chamomilla*(Kováčik et al., 2009a). In this work, interaction of salicylic acid and salinity on somesecondary metabolitesand physiological characteristics of *M. chamomile* were studied.

2. MATERIAL AND METHODS

Plant culture and growth conditions

This study was carried out in the Greenhouse of Fars Research Center for Agriculture and Natural Resources, Shiraz, Iran.fromNovember 2014 to September 2015. Seeds were sown in big plastic pots (30 × 50 cm). Each pot was filled with 10 kg of air-dried soil and five seedlings were used per pot for all treatments and were allowed to grow in the greenhouse with the mean day/night temperature and relative humidity of 29±4 °C, 38±5% RH and 17±2 °C, 50±5% RH respectively. AfterFourteenweeks, plants were subjected to differentlevels of salinity supplied with irrigation water. In order to preventosmotic shock, salt solutions were added gradually at several stagesand so, lasting for three weeks. To keep the levels of soil salt concentrationconstant, distilled water was used in subsequent irrigations. Plants (two and one weeks before flowering)were subjected to different levels of salicylic acid [control, 150, 300, and 450 mg l^{-1}].All SA solution treatments were sprayed to the shoots uniformly using a hand pump sprayer. Plants were harvested at full flowering stage and then shade dried.

Essential oil extraction

The plants were dried at room temperature (20-25°C). The essential oil of all dried samples (50g) was isolated by hydro-distillation for 3 h, using aClevenger-type apparatus according to the methodrecommended in British Pharmacopoeia (EuropeanPharmacopoeia, 2005).This work was repeated thrice. An average of the oils yieldwas calculated.

Preparation of crude extract

The sample was first ground to fine powder.Methanolic extract of the plant was

prepared asfollows: 20 g dry plant was macerated in 100 mLmethanol for 24 h. The extract was filtered andthen the below solution was concentrated in vacuumat 40 °C using a rotary evaporator.

Determination of antioxidant activity usingDPPH

The antioxidant activity of plant extract and thestandard antioxidant were assessed on the basisof radical scavenging effect of the stable DPPHfree radical. In a modified assay 28, 200 µl of a 40mg/L solution of DPPH radical in methanol wasmixed with 20 µl of 6.25-3200 µg/ml extracts andGallic acid respectively, solutions were left at roomtemperature for 30 minutes. The DPPH radicalinhibition was measured at 517 nm by using amicro-plate reader model biotek ELx808. The $IC_{50}$of each sample (concentration in µg/ml requiredto inhibit DPPH radical formation by 50 %) wascalculated by Matlab software. The antioxidantactivity is given by:100- [(sample Absorbance -blank Absorbance)× 100/ control Absorbance].The IC_{50} value for sample, defined as theconcentration of the test sample leading to 50 %reduction of the initial DPPH concentration, wascalculated from the nonlinear regression curveof Log concentration of the test extract (µg/ml)against the mean percentage of the radical scavengingactivity.Lower IC_{50}values correspond to higher antioxidant activities of the plant extract(Patro et al., 2005).

Extraction of polyphenol and HPLC analysis

The procedure for extraction of polyphenols wascarried out according to the modified method establishedby Justesen et al. Reference standardof 8 polyphenols (eugenol, sinapic acid,ellagic acid, catechin,,hesperidin,quercetin, , chloregenic acid andcaffeic acid)were purchased from Merck (Darmstadt, Germany).HPLC analysis was carried out on anAgilent 1200 series, equipped with a ZorbaxEclipse XDB-C_{18} column (4.6 × 5 µmi.d.; × 150mm film thickness, RP), and a photodiode arraydetector (PDA). Elution was monitored at 280and 320 nm. The column temperature was 30C^{o}.The injection volume was 20 µL and it was doneautomatically using auto-sampler. The total runningtime was 30 min. Gradient elution was selectedto achieve maximum separation and sensitivity.The elution was performed by varying theproportion of solvent A (formic acid 1 % in deionizedwater) to solvent B (Methanol (v/v)) as follows:Methanol: formic acid 1% (10:90), at 0 min;Methanol: formic acid 1% (25:75), at 10 min;Methanol: formic acid 1% (60:40), at 20 min andfinally, Methanol: formic acid 1% (70:30), at 30min

Proline analysis

Proline content was extracted from the leaf and flower tissues by themethod described by Bates et al. (1973). One hundred milligramsof dry leaf material was homogenized in 10 ml of 3% aqueous sul-fosalicylic acid and filtered through Whatman's No.1 filter paper.Two milliliters of the filtrate was mixed with 2 ml of acid-ninhydrinand 2 ml of concentrated acetic acid in a test tube. The reactionmixture was extracted with 4 ml toluene, and the absorbance wasmeasured at 520 nm with a UV Jenway 6300. L-proline was used asthe standard proline.

RESULTS AND DISCUSSION

Essential oil content

The EO yields of chamomile under different Nacl × SAtreatments are presented in Fig.1. The results indicatedthat $Nacl_{50,100} \times SA_0$significantly increased theEO content of chamomile as compared to control and other treatments ($p \leq 0.05$). Thepercentage of EO in different Nacl × SA treatmentsvaried from 0.46to 0.12 according to theapplied treatments. The maximum EOcontent (0.46%) was obtained by application of $Nacl_{100} \times SA_0$treatmentand the minimum EO content (0.12%) was obtained by application of $Nacl_0 \times SA_{150}$ treatment (Fig.1).The essential oil content was strongly increased in moderate salt-treated plants.In contrast, the EO components of *Nepetacataria*and *Menthapiperita*(Saharkhizand Goudarzi, 2014) were not significantly changed by application of different SA treatments. Visible increase of biomass was also detected in chamomile plants continuously cultivated in

medium enriched by 2 mM SA or with low concentration of SA (250 µM) (Kovačik et al. 2009b). The similar influence of variable doses of SA on growth can be explained by different mode of application, but the time of exposure and interaction with salt has also important impact.

Proline and Chlorophylls Content

The proline content of plants was significantly increased atdifferent Saltconcentrations (Fig. 2) The maximum amount of proline was observed in $Nacl_{100}\times SA_{300}$but its concentrationwas significantly decreased in all treatments of $Nacl_0\times SA_{100,300,\ 450}$concentrations (Fig. 2). Proline is known to serve as compatible osmolytes, protection of macromolecules and also as scavengers of reactive oxygenspecies under stressful conditions (Ashraf and Foolad, 2007).It is one of the most important amino acidthought to be involved in the protection against environmentalstresses, e.g., preventing lipid peroxidation (Mehtaand Gaur 1999), sowhen proline content was increased it was concluded that the planthas under stress effects.In line,explants of *Simmondsiachinensis* contained higher prolineamount up to 3 months of NaCl treatment (Roussos andPontikis 2003).Accumulation of total amino acids wasstimulated in NaCl-treated roots, especially due to exceptionalincrease of proline in *M. chamomilla*(Kovacik et al., 2009b).Proline synthesis is implicated asa mechanism of alleviating cytoplasmic acidosis and may maintain NADP+/NADPH ratios at values compatible with metabolism(Hare and Cress, 1997). Rapid catabolism of proline upon relief ofstress may provide reducing equivalents that support mitochon-drial oxidative phosphorylation, the generation of ATP for recoveryfrom stress and the repair of stress-induced damage (Hare andCress, 1997). The amount of chlorophyll a,b increased in moderate salinity so the chlorophyll a was significantly increased in $Nacl_{50}\times SA_{150,\ 300}$ and the maximum chlorophyll b content was obtained by application of $Nacl_{50}\times SA_{300}$ treatment(Fig. 3,4). With the increase in concentration of Nacl and foliar applied SA_{450}, the significant decrease in chlorophylls content was detected. SA exhibited both growth-promoting and growth-inhibiting in low and high concentration respectively, the latter being correlated with decrease of chlorophylls, water content and soluble proteins (Kovačik et al. 2009a). Moreover, duringsalt and other stressROS can be generated in thechloroplast by direct transfer of excitation energyfrom chlorophyll to produce singlet oxygen, or byunivalent oxygen reduction at photosystem I, in theMehler reaction (Allen, 1995).

Antioxidant activity (DPPH microplatemethod)

Various levels of different Nacl×SAconcentrations had significant effect on antioxidant activity (Fig. 5). It was determined by evaluating the effects of leafextracts on DPPH free radical scavenging activities at all Nacl×SAtreatments.At $Nacl_{150}\times SA_{300}$ and$Nacl_{100}\times SA_0$leaf extracts with the IC_{50} value of 1584.7 and 1595.6 µg ml^{-1}displayed the highest DPPH free radical quenching activity as comparedto other treatments (Fig.5). These effects may be related to the highertotal phenolic contents in chamomile under 150, 100 mg l^{-1} NaCltreatments (Table 1).Phenolic compounds exhibit antioxidant activity by inactivating lipid free radicals or by preventing the decomposition of hydroperoxides into free radicals (Pokorny et al., 2001)..It seems that in moderate Nacl was increased antioxidant activity. The maximum amount of DPPH free radicals were observed in $SA_{450}\times Nacl_0$ treatment (IC_{50} 2971.7 µg ml^{-1}) (Fig. 5). In terms of phenolic metabolism, it seems that the higher SA dose has a toxic effect, based on the sharp increase in phenylalanine ammonia-lyase (PAL) activity (Kovačik et al. 2009a).In the case of salinity, superoxide radicalwas found to play a crucial role in signaling the NaClinducedupregulation of antioxidative enzyme activitiesand stress tolerance (Vital et al. 2008). Oxidative stress-related parameters, such as hydrogen peroxide accumulation and activity of antioxidative enzymes, may be affected by SA exposure. The effect of exogenous SA depends on numerous factors, including the species and developmental

stage, the mode of application, and the concentration of SA(Vanacker et al. 2001; Horva´th et al. 2007).

Polyphenols

The amount of 8 investigatedpolyphenols are listed in table 1. Thehighest amount of polyphenols were eugenol, sinapic acid and ellagic acid, respectively. The maximum amount of eugenol, ellagic acid, catechin, hesperedin andquercetin were observed in$Nacl_{150} \times SA_{300}$ treatment. The maximum sinapic acid (307.1mg l^{-1}) was obtained by application of $Nacl_{150} \times SA_0$ treatmentand the maximumchloregenic acid(66.55 mg l^{-1}) andcaffeic acid (9.93 mg l^{-1}) were obtained by application of $Nacl_{50} \times SA_{300}$ andof $Nacl_{50} \times SA_0$ treatment respectively (Table 1).The increase in phenolic contents in different plant tissues under increasing salinity has also been reported in a number of plants (Muthukumarasamy et al., 2000). Navarro et al. (2006) reported an increase in total phenolic content in red pepper plants under moderate salinity levels. Thehigher SA dose has a toxic effect, which is followed by an increase intotal soluble phenolics, lignin accumulation and themajority of the 11 detected phenolic acids(Kovačik et al. 2009b).Among phenolic acids,accumulation of protocatechuic acid was the most enhancedin NaCl-exposed leaf rosettes whilechlorogenicand caffeic acids in the roots.Total soluble phenols increased after NaCl and SAtreatments, but root lignin content was not affected.Roussos and Pontikis (2003) found decrease of total phenols and some of fourphenolic acids in jojoba explants after 3 months of exposureto NaCl, indicating attenuation of phenolic metabolismafter long-term presence of NaCl stress.

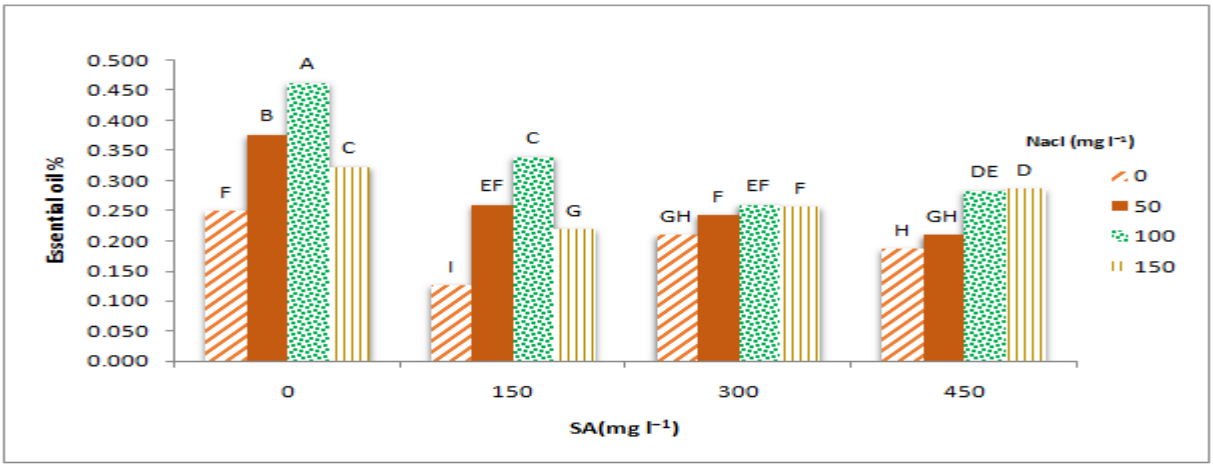

Fig 1. Effect of interaction of salicylic acid and salinity on essential oil content

Values followed by the same small letter did not share significant differences at 5% (Duncan's test).

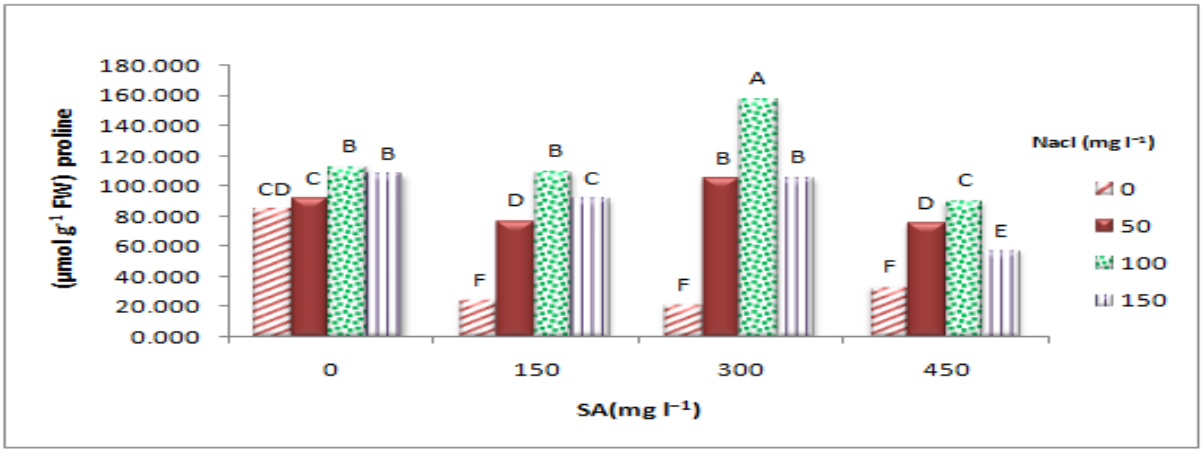

Fig 2. Effect of interaction of salicylic acid and salinity onproline content

Values followed by the same small letter did not share significant differences at 5% (Duncan's test).

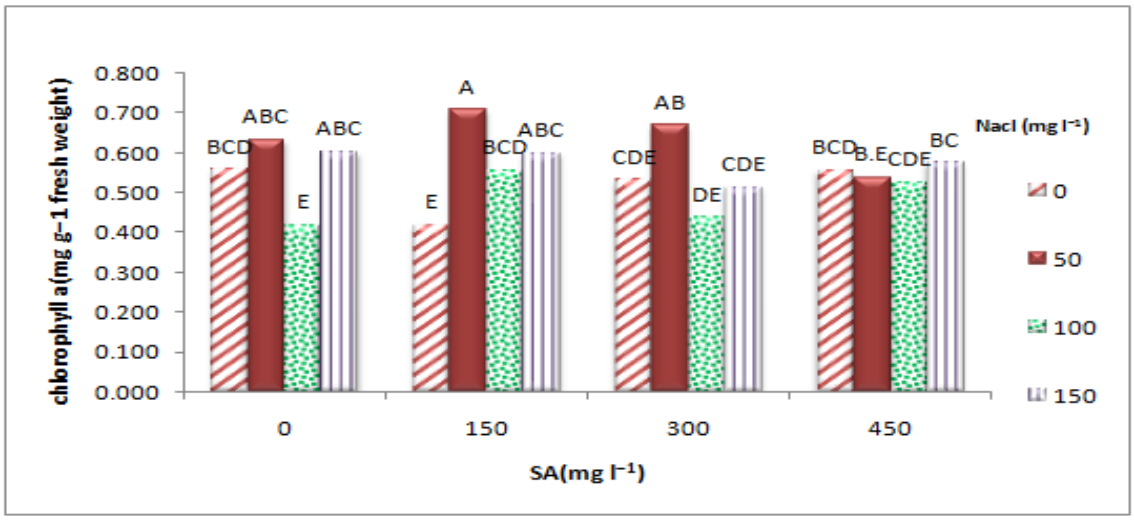

Fig 3. Effect of interaction of salicylic acid and salinity onchlorophyll a

Values followed by the same small letter did not share significant differences at 5% (Duncan's test).

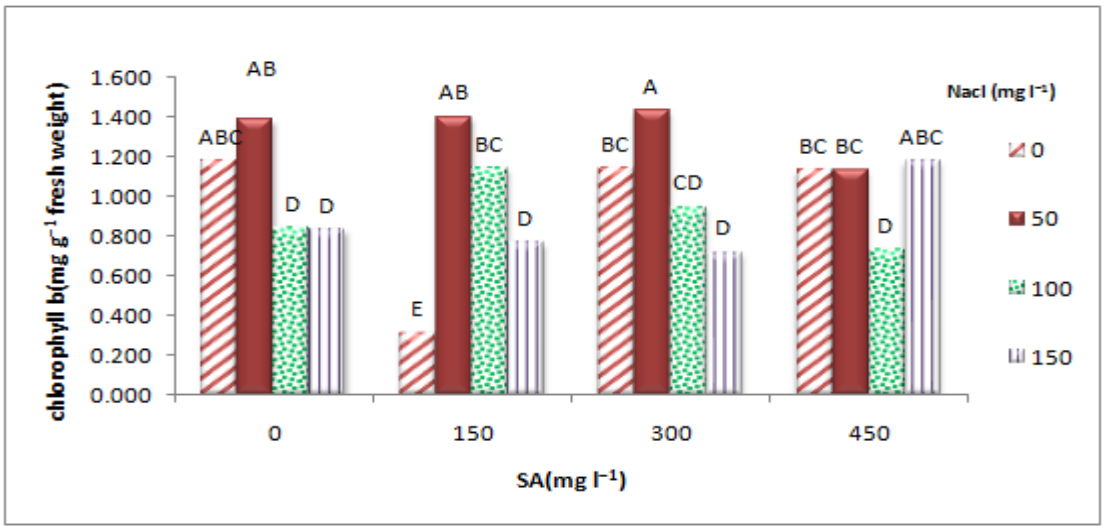

Fig 4. Effect of interaction of salicylic acid and salinity onchlorophyll b

Values followed by the same small letter did not share significant differences at 5% (Duncan's test).

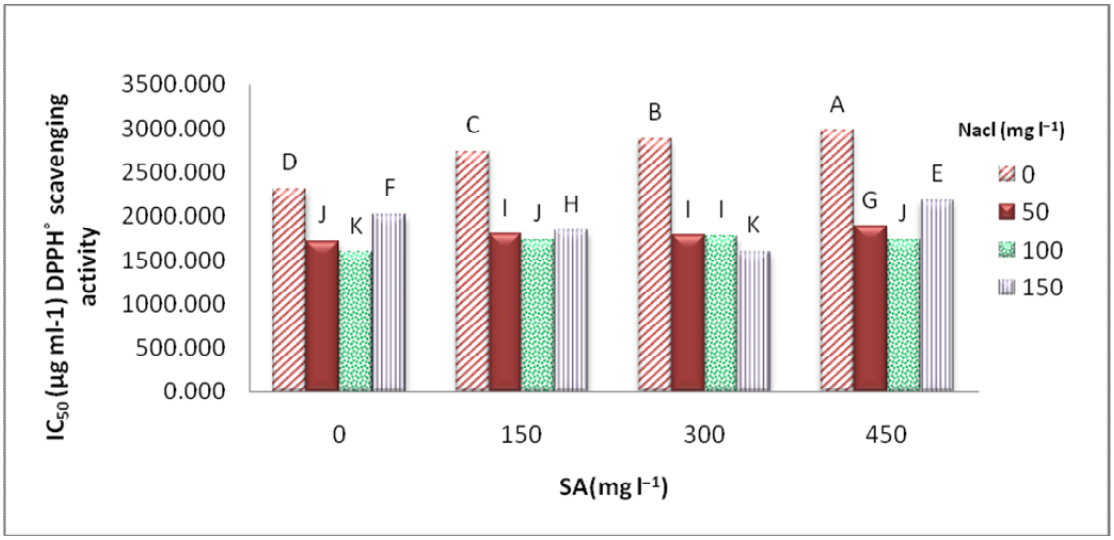

Fig 5. Effect of interaction of salicylic acid and salinity on antioxidant activity (IC50 values)

Values followed by the same small letter did not share significant differences at 5% (Duncan's test).

Table 1: Effect of interaction of salicylic acid and salinity on polyphenols content of *Matricariachammomile*.

tretment[a]	chloregenic acid	caffeic acid	catechin	sinapic acid	hesperedin	ellagic acid	quercetin	eugenol
$Nacl_{50}$ SA_0	62.70	9.93	57.69	291.91	25.13	111.11	21.498	217.73
$Nacl_{50}$ SA_{150}	43.53	4.35	39.80	232.14	24.98	107.24	20.28	202.97
$Nacl_{50}$ SA_{300}	66.55	1.05	56.09	256.16	33.39	122.60	21.85	245.51
$Nacl_{50}$ SA_{450}	34.483	0.98	49.31	209.33	29.54	121.20	23.34	264.31
$Nacl_{100}$ SA_0	56.21	1.34	52.62	289.45	31.59	122.15	26.79	287.56
$Nacl_{100}$ SA_{150}	47.76	ND	54.17	203.75	26.43	97.21	16.33	300.33
$Nacl_{100}$ SA_{300}	48.87	1.26	56.36	264.43	33.14	121.11	23.73	270.12
$Nacl_{100}$ SA_{450}	39.88	6.02	41.02	199.42	20.45	86.42	14.51	166.12
$Nacl_{150}$ SA_0	54.18	ND	55.06	307.01	25.74	125.56	21.10	269.03
$Nacl_{150}$ SA_{150}	55.77	4.64	62.12	250.00	32.68	114.69	28.48	217.53
$Nacl_{150}$ SA_{300}	65.72	7.96	63.55	294.99	41.88	181.60	41.82	311.63
$Nacl_{150}$ SA_{450}	34.46	2.45	32.33	160.76	18.35	90.05	16.13	169.40
$Nacl_0$ SA_{150}	51.10	4.49	47.76	271.89	27.65	134.14	18.57	251.39
$Nacl_0$ SA_{300}	30.41	6.82	32.51	254.90	21.74	114.14	19.65	228.66
$Nacl_0$ SA_{450}	26.71	5.58	32.75	223.61	19.25	98.25	17.62	189.71
$Nacl_0$ SA_0	38.69	3.54	41.10	165.07	19.80	111.58	17.52	215.41

ND: not detected

[a] Calculated mean amount of the polyphenol (mg l^{-1}) based on the weight of the ground dry plant in three replicates.

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