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Effect of Different Media and Salinity Levels on Growth Traits of Rosemary *(Rosmarinus officinalis* L.*)*

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Abstract: Rosemary (*Rosmarinus officinalis* L.*)* is a member of the Lamiaceae family, it is used for ornamental and many medicinal purposes. Increasing of saline lands and the shortage of agricultural lands, identification and improvement of salt tolerant plants particularly medical and ornamental is important. Purpose of this trial is investigation of different growth media and salinity effect on growth of rosemary (*Rosmarinus officinalis* L.). Pot experiment was carried out as a factorial experiment with completely randomized in three replications. Four levels of saline water included: tap water, saline water containing NaCl 100 mM, 150 mM and Caspian Sea water. Applied different media were loam soil (b_1 or control), b_2(control medium and Azolla compost 1:1), b_3 (peanut cocoon and control medium 1:1) and the fourth media b_4(40% Azolla compost, 30%peanut cocoon and rice husk 30%). Salinity treatment was applied for three weeks and some growth parameters were measured. The results showed that the interaction of salinity and media influenced significantly ($p<0.05$) plant height, number of lateral branches, root dry weight and root length but the factors interaction did not have significant effect on number of leaves and root fresh weight. Interaction of salinity of Caspian Sea water and media"40% Azolla compost, 30%peanut cocoon and rice husk 30% is reducing growth rosemary.

Key words: Rosemary · Salinity stress · Azolla compost · Caspian Sea

INTRODUCTION

Rosemary, *Rosmarinus officinalis* L, is a member of mint family Lamiaceae. It is endemic to the Mediterranean regions and commonly grown in many parts of Iran [1]. Rosemary is an aromatic evergreen shrub that its abundant branches are soft and fluffy when young and leaves are thin and mutual. Application of plants and herbs is a conventional remedy for treating diseases in large parts of the world, especially in advanced countries. Medicinal plants can compensate the common disadvantages of using antibiotics. The essence of plants including Rosemary is reported to have anti-microbial properties [2,3]. Rosemary is anti emphysema, has disinfectant properties and increases the secretion of digestive juices and bile and also blood circulation. It also works for treating rheumatism and migraine. Rosemary is used in the production of cologne and as deodorant in shampoos, creams, soaps and cosmetics [1]. Nowadays, salinity stress, besides drought, is one of the environmental factors limiting agricultural production. According to the latest statistics, 20% of lands in the world are faced with the problem of salinity. Salinity is a non-biological stress that limits the growth of plant; Decreased growth is an adaptation for survival of plant in stress condition [4]. As far as climate and geographical position in the field of medicinal plants growth are mentioned, Iran considered one of the best parts of the world [5].

Success in the production of medicinal plants like Rosemary lies in selecting appropriate substrate and condition. In addition to having desired physical, chemical and biological properties, a suitable substrate should be available and relatively inexpensive, stable and light enough to be easy to use and its transport be affordable [6]. The organic wastes from agriculture are used in the culture of ornamental flowers and plants. They are used in form of compost and mostly have appropriate physical and chemical characteristics. Rice husk is used in agriculture as fertilizer and an additive to soil. The quality of Bran depends on the amount of phosphorus oxides, potassium, calcium and organic nitrogen. In addition to providing organic matter and compounds containing phosphorus for soil, Bran also acts as a

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thinner. Agricultural products have a better growth in salty soils in the presence of rice husk. It also increases the rate of water infiltration in soil [7]. Azolla compost that is obtained from the Fern of composted Azolla can hold water at a high volume and correct physical and chemical properties of soil. It also paves the way for the growth of beneficial soil organisms and bacteria [8]. Peanut cocoon, which is discarded as waste each year, can be used as substrate with soil. Due to the volume of production of these materials in the north of Iran, their practical use is subjected to the knowledge of their properties.

MATERIALS AND METHODS

This work aims to study the effects of different substrates and organic wastes and also salinity stress tolerance in Rosemary. This study was carried out in 2011 in Lahijan located in the east of Guilan Province. This city lies at 50°2'E 27°15'N and is 2 meters above sea level. Factorial with a randomized complete blocks plan was used in this study. Two factors of salinity (A) and substrate (B) in 4 levels and with 3 replicates for each were studied.

At first, 4 types of substrates were prepared for 144 vases. Garden soil was used as substrate b_1, substrate b_2 was a combination of garden soil and Azolla compost with a ratio of 1:1, peanut cocoons were mixed with garden soil to form substrate b_3 and substrate b_4 was a combination of rice husk (40%), Azolla compost (30%) and peanut cocoons (30%). There were 4 treatments for salinity including tap water (a_1), 100 mM NaCl solution (a_2), 150 mM NaCl solution (a_3) and water of the Caspian Sea (a_4) which were exercised in form of irrigation three times a week for three weeks. Different cuttings were obtained from the original Rosemary and grown in a greenhouse located in Karaj. Cuttings rooted within 2 months and 144 of them which had the same size were selected. Each of rooted cuttings was planted in rubbery vases containing the desired substrate. Vases were put in three rows of 48 in outdoors. Irrigation was done three times a week with no fertilizing and spraying. Chemical properties of substrates including total nitrogen, available potassium, available phosphorus, EC and pH were measured. Kjeldahl and Olson methods were used to measure total nitrogen and available phosphorus, respectively and available potassium was measured through extraction with ammonium acetate in a neuter environment and reading flame photometer. Samples of substrates were mixed with distilled water (1:5 ratio), shook for 30 minutes by shaker device, passed through filter paper and finally EC and pH measured by model HI 96304 EC meter and pH meter model HI 98107 (1). Growth indices including plant height, number of branches, number of new leaves, root length, number of roots, wet weight and dry weight were measured at the end of this period. Variance analysis of obtained data was done by SPSS software and Tukey test was used to compare means at 1% and 5% level of likelihood.

RESULTS

Plant Height: The results of variance analysis of plant height show that the effect of salinity (A) and also substrate (B) at 1% level of likelihood and the mutual effect of salinity and substrate at 5% level of likelihood are statistically significant (Table 1). In terms of salinity factor (A), mean comparison results show that height was maximum in plants treated with a_1 (tap water) (25.3 cm) and in those treated with a_4 (the Caspian Sea water) was minimum (18.25 cm). When it comes to the type of

Table 1: Variance analysis of the effect of salinity and substrate on height, number of branches and number of leaves in Rosemary

		Mean squares		
SOV	df	Height	Number of branches	Number of leaves
Replicate (R)	2	2.98*	0.25^{ns}	30.64^{ns}
Salinity (A)	3	243.56**	1.74**	136.68**
Substrate (B)	3	117.51**	8.02**	332.52**
Salinity (A) ×Substrate (B)	9	1.94*	0.72*	27.07^{ns}
Experimental error	30	0.75	0.25	17.51
(%) Coefficient of variation		4.03	34.78	47.26

Show non-significant and significant statistical difference at 1% and 5% level of likelihood**, *ns,

		MS		
SOV	df	Height	No. of leaves	No. of branches

Table 2: Variance analysis of the effect of salinity and substrate on the amount of chlorophyll in Rosemary

		Mean squares		
Changes	Degrees of freedom	Chlorophyll a	Chlorophyll b	Total Chlorophyll
Replicate (R)	2	0.09^{ns}	0.01^{ns}	0.17^{ns}
Salinity (A)	3	74.12**	90.16**	5.03**
Substrate (B)	3	61.96**	21.07**	153.49**
Salinity (A) ×Substrate (B)	9	1.12**	1.47**	0.81**
Experimental error	30	0.12	0.19	0.13
(%) Coefficient of variation		3.56	9.34	2.56

Show non-significant and significant statistical difference at 1% and 5% level of likelihood**, *ns,

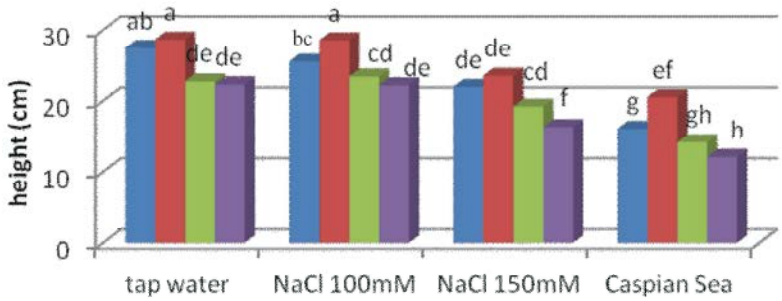

Fig. 1: Mean comparison of plant height in the interaction of different levels of salinity (A) and substrate (B)
b_1: garden soil; b_2: garden soil and Azolla compost; b_3: peanut cocoons and garden soil; b_4: rice husk (40%), Azolla compost (30%) and peanut cocoons (30%)

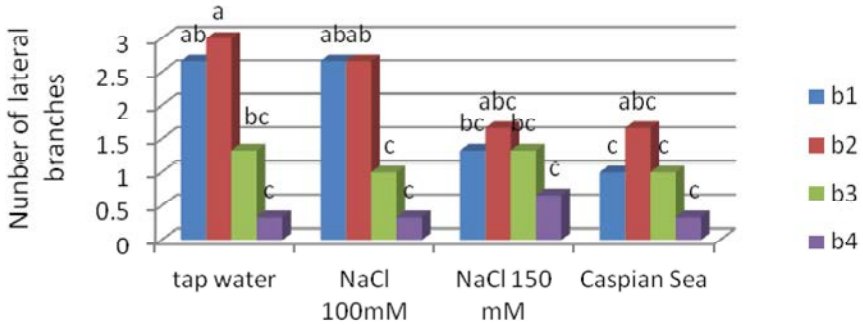

Fig. 2: Mean comparison of the number of new branches in the interaction of different levels of salinity (A) and substrate (B)
b_1: garden soil; b_2: garden soil and Azolla compost; b_3: peanut cocoons and garden soil; b_4: rice husk (40%), Azolla compost (30%) and peanut cocoons (30%)

substrate (B), the maximum and minimum plant height were observed in b_2 treatment (mixture of garden soil and Azolla compost) (25.33 cm) and b_4 treatment (combination of rice husk, Azolla compost and peanut cocoons) (18.25), respectively (Tables 8 and 9). About the mutual effect of these two factors, the maximum plant height was observed in the mutual effect of b_2 and a_1 (28.66 cm) and the minimum in b_4 and a_4 interaction (12.11 cm) (Fig. 1).

Number of New Branches: The results of variance analysis of the number of new branches show that the effect of salinity (A) and also substrate (B) at 1% level of likelihood and the mutual effect of salinity and substrate at 5% level of likelihood are statistically significant (Table 1). According to mean comparison, the maximum and minimum number of new branches was observed in a_1 (tap water) (1.83) and a_4 (water of the Caspian Sea) (1) treatments of salinity, respectively. Among substrate treatments, the maximum and minimum number of new branches belonged to b_2 (2.25) and b_4 (0.41), respectively (Tables 8 and 9). When it comes to the mutual effect, the maximum number of new branches was obtained in the interaction of b_2 and a_1 with a average of 3, while the interaction of b_4 with a_1 and a_2 showed the minimum with a average of 0.33 (Fig. 2).

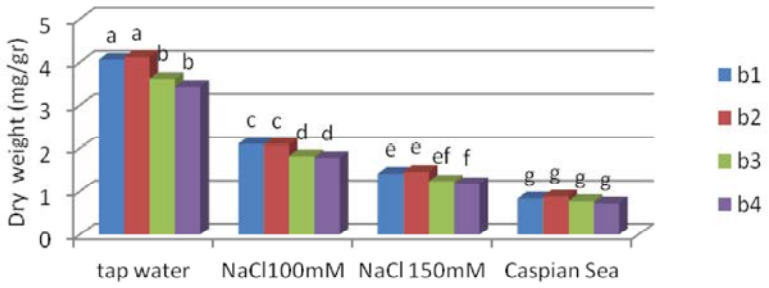

Fig. 3: Mean comparison of dry weight of shoot in the interaction of different levels of salinity (A) and substrate (B) b_1: garden soil; b_2: garden soil and Azolla compost; b_3: peanut cocoons and garden soil; b_4: rice husk (40%), Azolla compost (30%) and peanut cocoons (30%)

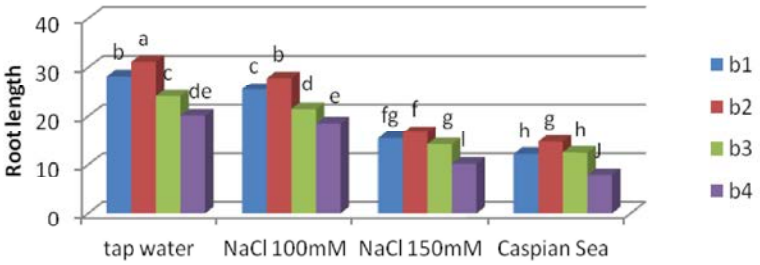

Fig. 4: Mean comparison of root length in the interaction of different levels of salinity (A) and substrate (B) b_1: garden soil; b_2: garden soil and Azolla compost; b_3: peanut cocoons and garden soil; b_4: rice husk (40%), Azolla compost (30%) and peanut cocoons (30%)

Dry Weight of Shoot: The results of variance analysis of dry weight of shoot show that the effect of salinity (A), substrate (B) and the mutual effect of them are statistically significant at 1% level of likelihood (Table 3). Mean comparison results show that the maximum and minimum dry weight of shoot, among different treatments of salinity, belongs to a_1 with an average of 3.81 g and a_4 with an average of 0.81 g, respectively. In terms of substrate, the highest dry weight of shoot was observed in b_2 with a average of 2.11 g and the lowest was obtained from b_4 with a average of 1.79 g (Tables 7 and 11). About the mutual effect of salinity and substrate, the interaction of b2 and a1 showed the highest figures of dry weight of shoot with an average of 4.06 g and the interaction of b4 and a4 showed the lowest figures with an average of 0.73 g (Fig. 3).

Root Length: The results of variance analysis of dry weight of shoot show that the effect of salinity (A), substrate (B) and the mutual effect of them are statistically significant at 1% level of likelihood (Table 3). In terms of salinity (A), a1 with an average of 25.84 cm and a4 with an average of 11.79 cm had the highest and the lowest root length, respectively. When it comes to substrate treatments, b2 showed the highest root length with an average of 22.53 cm, while those planted in b4 showed the lowest figures with an average of 14.20 cm (Tables 7 and 11). The interaction of b2 and a1 with an average of 31.11 cm and the interaction of b4 and a4 with an average of 7.94 cm showed the highest and the lowest figures of root length respectively (Fig. 4).

Number of Roots: The results of variance analysis of the number of roots show that the effect of salinity (A) and also substrate (B) at 1% level of likelihood and the mutual effect of salinity and substrate at 5% level of likelihood are statistically significant (Table 3). Mean comparison results show that the highest number of root belonged to a_1 with an average of 11.42 and the lowest number was observed in a_4 with an average of 5.33, among different salinity treatments. As far as substrate in concerned, the highest and the lowest number of root were recorded from b_2 with an average of 11.50 and b_4 with an average of 5.83, respectively (Tables 7 and 11). In terms of mutual effect of salinity (A) and substrate (B), the highest number of root was observed in the interaction of b_2 and a_1 with an average of 14.67 and the lowest figures were obtained from the interaction of b_4 and a_4 with an average of 2.66 (Fig. 5).

Table 3: Variance analysis of the effect of salinity and substrate on wet weight, dry weight, root length and number of root in Rosemary

Changes	Degrees of freedom	Mean squares Root length	Number of root	Wet weight	Dry weight
Replicate (R)	2	0.04^{ns}	0.77^{ns}	1.06^{ns}	0.005^{ns}
Salinity (A)	3	556.67**	77.07**	669.89**	20.59**
Substrate (B)	3	151.44**	69.57**	123.72**	0.36**
Salinity (A) ×Substrate (B)	9	5.09**	1.03*	2.74^{ns}	0.03**
Experimental error	30	0.38	0.37	1.53	0.007
(%) Coefficient of variation		3.32	7.15	3.07	4.26

Show non-significant and significant statistical difference at 1% and 5% level of likelihood$_{**}$, $_{*}$ns,

Table 4: Variance analysis of the effect of salinity and substrate on sodium, potassium and calcium content in Rosemary

Changes	Degrees of freedom	Mean squares Na (%)	K (%)	Ca (%)
Replicate (R)	2	0.30*	0.01^{ns}	0.01^{ns}
Salinity (A)	3	134.78**	4.40**	3.22**
Substrate (B)	3	10.46**	0.21**	0.06**
Salinity (A) ×Substrate (B)	9	0.38**	0.01^{ns}	0.01^{ns}
Experimental error	30	0.09	0.02	0.01
(%) Coefficient of variation		4.15	17.07	11.84

Show non-significant and significant statistical difference at 1% and 5% level of likelihood$_{**}$, $_{*}$ns,

Table 5: Mean comparison of the effect of different levels of salinity on height, number of branches and number of new leaves in Rosemary

Treatment	Plant height (cm)	Number of shoot	Number of new leaves
tap water (a_1)	25.30a	1.83a	11.67a
NaCl 100mM solution (a_2)	24.94a	1.66ab	11.67a
(a_3) NaCl 150mM solution	25.70b	1.25bc	7.16b
Water of the Caspian Sea (a_4)	15.57c	1.00c	4.91b

Similar letters in each column indicates that there is no significant difference between the average of treatments

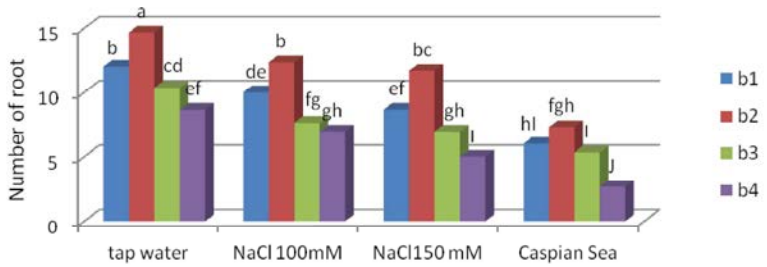

Fig. 5: Mean comparison of the number of root in the interaction of different levels of salinity (A) and substrate (B) b_1: garden soil; b_2: garden soil and Azolla compost; b_3: peanut cocoons and garden soil; b_4: rice husk (40%), Azolla compost (30%) and peanut cocoons (30%)

Chlorophyll: Variance analysis of data (Table 2) shows that the amount of chlorophyll a, chlorophyll b and total chlorophyll is statistically significant at 1% level of likelihood. Mean comparison of data shows that the maximum of chlorophyll a belonged to a_1 with an average of 12.16 mg/g and its minimum was observed in a_4 with an average of 6.67 mg/g indicating a decrease by two times. The highest and the lowest amount of chlorophyll b were observed in a_1 with a average of 8.15 mg/g and a_4 with an average of 2.21, respectively. This shows that the amount of chlorophyll b decreased by four times. The amount of total chlorophyll showed its maximum in a_1 with an average of 20.31 mg/g, while the minimum amount was observed in a_4 with an average of 8.89 mg/g. Among different treatments of substrate (B), the highest and the lowest amount of chlorophyll a was observed in b_2 with

Table 6: Mean comparison of the effect of different levels of salinity on the amount of chlorophyll in Rosemary

Treatment	Chlorophyll a	Chlorophyll b	Total Chlorophyll
Tap water (a_1)	12.16a	8.15a	20.31a
NaCl 100mM solution (a_2)	11.41b	5.60b	17.01b
c NaCl 150mM solution (a_3)	9.01c	2.79c	11.81c
Water of the Caspian Sea (a_4)	6.67d	2.21d	8.89d

Similar letters in each column indicates that there is no significant difference between the average of treatments

Table 7: Mean comparison of the effect of different levels of salinity on wet weight, dry weight, root length and number of root in Rosemary

Treatment	Wet weight of shoot (g)	Dry weight of shoot	Root length	Number of root
Tap water (a_1)	48.86a	3.81a	25.84a	11.42a
NaCl 100mM solution (a_2)	43.69b	0.89b	23.32b	9.25b
(a_3) NaCl 150mM solution	37.79c	1.33c	14.05c	8.08c
Water of the Caspian Sea (a_4)	31.14d	0.81d	11.79d	5.33d

Similar letters in each column indicates that there is no significant difference between the average of treatments

Table 8: Mean comparison of the effect of different levels of salinity on sodium, potassium and calcium content in Rosemary

Treatment (%)	Na (%)	K (%)	Ca
tap water (a_1)	4.03d	1.66a	1.57a
NaCl 100mM solution (a_2)	5.17c	0.94b	0.89b
(a_3) NaCl 150mM solution	8.22b	0.47c	0.57c
Water of the Caspian Sea (a_4)	11.51a	0.31d	0.40d

Similar letters in each column indicates that there is no significant difference between the average of treatment

Table 9: Mean comparison of the effect of different substrates on height, number of branches and number of new leaves in Rosemary

Treatment	Plant height (cm)	Number of shoot	Number of new leaves
Garden soil (b_1)	22.77b	1.91a	12.58a
garden soil and Azolla compost (b_2)	25.33a	2.25a	12.58a
peanut cocoons and garden soil (b_3)	19.91c	1.16b	8.83a
rice husk (40%) + Azolla compost (30%)+ peanut cocoons (30%) (b_4)	18.25d	0.41c	1.41b

Similar letters in each column indicates that there is no significant difference between the average of treatments

Table 10: Mean comparison of the effect of different substrates on the amount of chlorophyll in Rosemary

Treatment	chlorophyll a	chlorophyll b	chlorophyll Total
garden soil (b_1)	11.11b	5.10b	16.21b
garden soil and Azolla compost (b_2)	12.17a	6.34a	18.51a
peanut cocoons and garden soi (b_3)	8.90c	4.00c	12.90c
rice husk (40%)+ Azolla compost (30%)+ peanut cocoons (30%) (b_4)	7.08d	3.31d	10.40d

Similar letters in each column indicates that there is no significant difference between the average of treatments

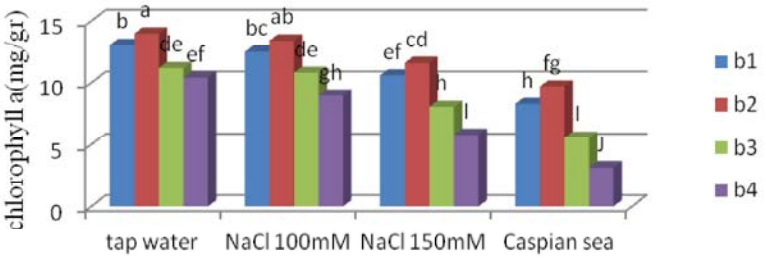

Fig. 6: Mean comparison of chlorophyll a in the interaction of different levels of salinity (A) and substrate (B) b_1: garden soil; b_2: garden soil and Azolla compost; b_3: peanut cocoons and garden soil; b_4: rice husk (40%), Azolla compost (30%) and peanut cocoons (30%)

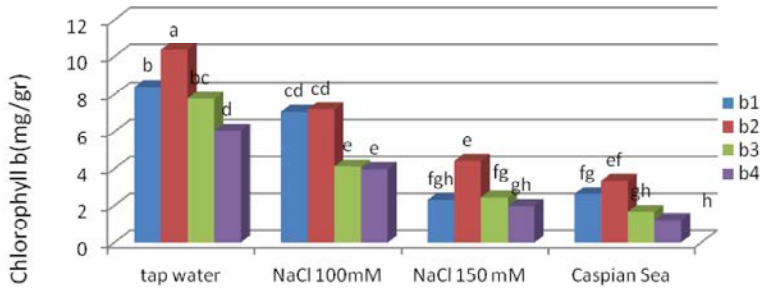

Fig. 7: Mean comparison of chlorophyll b in the interaction of different levels of salinity (A) and substrate (B) b_1: garden soil; b_2: garden soil and Azolla compost; b_3: peanut cocoons and garden soil; b_4: rice husk (40%), Azolla compost (30%) and peanut cocoons (30%)

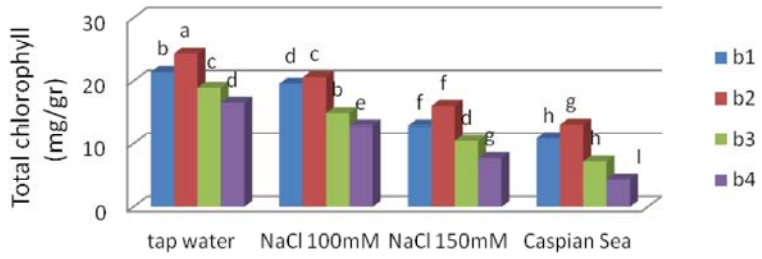

Fig. 8: Mean comparison of total chlorophyll in the interaction of different levels of salinity (A) and substrate (B) b_1: garden soil; b_2: garden soil and Azolla compost; b_3: peanut cocoons and garden soil; b_4: rice husk (40%), Azolla compost (30%) and peanut cocoons (30%)

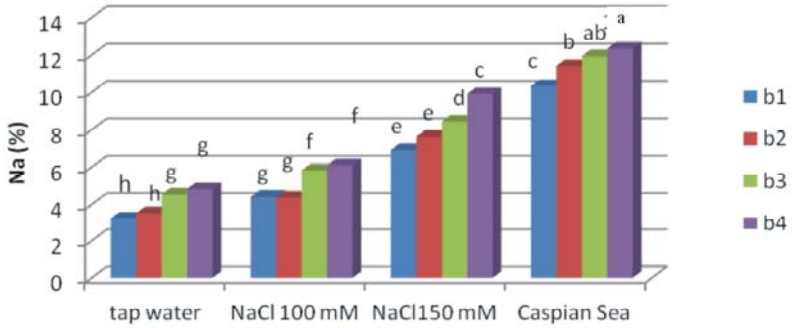

Fig. 9: Mean comparison of sodium content in the interaction of different levels of salinity (A) and substrate (B) b_1: garden soil; b_2: garden soil and Azolla compost; b_3: peanut cocoons and garden soil; b_4: rice husk (40%), Azolla compost (30%) and peanut cocoons (30%)

an average of 11.11 mg/g and b_4 with average of 7.08 mg/g, respectively. In terms of chlorophyll b, the maximum amount belonged to b_2 with an average of 6.34 mg/g and the minimum was observed in b_4 with an average of 3.31 mg/g indicating a decrease by two times. The highest and lowest amount of total chlorophyll were observed in b_2 with a average of 18.51 mg/g and b_4 with an average of 10.40 mg/g, respectively. As far the mutual effect of salinity and substrate is mentioned, the highest amount of chlorophyll a, chlorophyll b and total chlorophyll were obtained in the interaction of b_2 and a1 with an average of 13.97, 10.37 and 24.34 mg/g, respectively. Also, the lowest amount of them was observed in the interaction of b_4 and a_4 with an average of 3.15, 1.22 and 4.38 mg/g, respectively. Given the above results, it can be concluded that chlorophyll a, chlorophyll b and total chlorophyll decreased by five, ten and six times, respectively (Fig. 6, 7 and 8).

Sodium Content: Variance analysis results of sodium content show that the effect of salinity (A), substrate (B) and the mutual effect of them are statistically significant

Table 11: Mean comparison of the effect of different substrates on wet weight, dry weight, root length and number of root in Rosemary

Treatment	Wet weight of shoot	Dry weight of shoot	root length	number of root
garden soil (b_1)	43.07a	2.11a	20.28b	9.16b
garden soil and Azolla compost (b_2)	43.14a	2.14a	22.53a	11.50a
peanut cocoons and garden soi (b_3)	38.33b	1.87a	18.00c	7.58c
rice husk (40%)+ Azolla compost (30%)+ peanut cocoons (30%) (b_4)	36.94c	1.79a	14.20d	5.83d

Similar letters in each column indicates that there is no significant difference between the average of treatments

Table 12: Mean comparison of the effect of different substrates on sodium, potassium and calcium content in Rosemary

Treatment (%)	Na (%)	K (%)	Ca
garden soil (b_1)	6.22d	0.91ab	0.86b
arden soil and Azolla compost (b_2)	6.73c	0.99a	0.96a
peanut cocoons and garden soi (b_3)	7.68b	0.79bc	0.82b
rice husk (40%) + Azolla compost (30%)+ peanut cocoons (30%) (b_4)	8.30a	0.69c	0.79b

at 1% level of likelihood (Table 4). Mean comparison results showed that the maximum and minimum amount of sodium belonged to a_4 with an average of 11.51% and a_1 with an average of 4.03%, respectively. Among different treatments of substrate, b_4 and b_1 showed the highest and the minimum sodium content with an average of 8.30% and 6.22%, respectively (Tables 8 and 12). About the mutual effect of salinity and substrate, the highest of sodium content were observed in the interaction b_1 and a_1, while the lowest amount was obtained from the interaction of b_4 and a_4 (Fig. 9).

DISCUSSSION

The results of this study indicated that salinity is one of the environmental stresses which reduced the ability of roots to absorb water and also disrupts nutritional and biological processes of plant [9]. Leading saltwater of the Caspian Sea in Guilan Province, rice farms located in coastal strip incur substantial losses [10]. Decreased growth caused by salinity is due the competition of NaCl in nutrient uptake. When plant is subjected to salinity, the balanced flow of sodium, chloride and other ions like potassium and calcium transport will be disrupted [11]. Salinity damage in plant which is due to the osmotic effect is equal to the reduction of water, especial toxicity effect of ions and disruption in nutrient uptake [8]. Reduced height of Rosemary caused by salinity stress in the present study is consistent with the findings of Hassani [12] on Basil, El-Keltawi on Marjoram [13], Ibrahim *et al*. [14] on Coleus and sage and Pascal *et al* [9] on beans. It seems that reduced root length caused by salinity stress is due to the reduced photosynthesis. Also, Shannon [15] has reported that increased salinity in water and soil severely decreases the growth of aerial organ and stem of plants, thereby negatively affects the performance of plants.

Khalighi *et al*. [8] studied the effect of Azolla on composting the bark of trees, tea wastes and rice husk and reported that Azolla compost is rich in nutrients. They also stated that bark compost and its blend with Azolla provide the best properties for the substrate of ornamental plants. In a study by Premuzic *et al*. [11] on tomato, it was shown that fruits which had better quality were grown in organic substrates which had better qualitative and quantitative properties than mineral substrates [11]. The effect of organic matter improves the physical and chemical properties of soil and provides a better feed condition for it [16].

Hajar *et al*. [17] examined the effect of salinity treatments up to 300 mM of sodium chloride on Nigella. They concluded that root growth decreases by the increase of salinity up to 150 mM sodium chloride. Decreased number of leaves in salinity stress in the present study is consistent with previous studies done by Ghoulam *et al*. [18] on sugar beet. Also, the effect of salinity stress on different genotypes of Green Cumin caused decreased stem length, root length, wet and dry weight of root and wet and dry weight of stem [19].

Recommendations: Given the problem of saline soils, shortage of fresh water and plurality of saline water, finding plants like Rosemary which can be productive in saline waters and substrates is of importance but more research are needed.

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