American-Eurasian J. Agric. & Environ. Sci., 13 (1): 68-71, 2013
ISSN 1818-6769

DOI: 10.5829/idosi.aejaes.2013.13.01.1888

Effect of Different Salinity Levels on the Composition of Rosemary (*Rosmarinus officinalis*) Essential Oils

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Abstract: Crop production is limited by environmental stresses, their impact and severity. Amongst the environmental stresses, the adverse effects of salinity on crop production are more drastic. Identifying resistant plants to salinity is vital. The aim of this trial was to investigate the changes in the chemical composition of the essential oils of rosemary shoots (*Rosmarinus officinalis*) under different levels of water salinity. To study the effect of salinity, rosemary plants were treated in triplicate with four water samples at different levels of salinity: tap water (S_1), NaCl 100 mM solution (S_2), NaCl 150 mM solution (S_3) and water from the Caspian Sea (S_4). The results revealed that salinity significantly affects the essential oils of rosemary compounds. The highest and lowest percentage of oils were obtained with the S_4 (Caspian Sea water) and S_1 (tap water) treatments, respectively. The main constituents of the essential oils were 1,8-cineole, borneol, camphor, α-pinene and β-pinene under different levels of salinity.

Key words: *Rosmarinus officinalis* · Salinity stress · NaCl · Essential oil · Caspian Sea

INTRODUCTION

Environmental stress is a major factor determining the global patterns of plant distribution [1]. Salinity stress is one of these major factors limiting agricultural production, with soil salinity having the most adverse effects on crop production. According to recent reports, 20% land worldwide is subjected to salinity stress [2]. The total area of saline lands in Iran is about 24 Mha, which is about 15% of the total land in the country [3]. The availability of non-saline water for irrigation is limited and the water quality continues to decline in arid and semi-arid areas. Use of saline water in agriculture now seems inevitable. Although some plant species can tolerate saline conditions, most plants cannot tolerate even low salt concentrations in their root zone [4].

Medicinal plants are often used in the manufacture of synthetic drugs based on the chemical structures of the natural products [5]. One such medicinal plant that contains flavonoids and essential oils is rosemary (*Rosmarinus officinalis)*, a member of the Lamiaceae family that consists of more than 4000 species in 200 genera distributed worldwide. Rosemary is a fragrant, perennial evergreen plant. It is used for ornamental and many medicinal purposes. The plant is native to the Mediterranean region, Europe and the Near East [6]. Rosemary has been traditionally used to improve memory and as a paregoric. It is used as a stimulant, carminative, anti-spasmodic, diaphoretic and anti-emetic. Rosemary essential oils are known to contain anti-microbial compounds and are thus used in the manufacture of herbal shampoos to strengthen the skin and hair [7]. The highest concentration of essential oils is found in the leaves, but the stems and flowers also contain small amounts of these oils [8]. The primary components of leaves and flower shoots of rosemary are essential oils. 1,8-Cineole, camphor, α-pinene, β-pinene and borneol are active constituents of rosemary essential oils [9]. The concentration of these constituents varies depending on location and altitude of the field. The concentration and types of constituents of rosemary essential oils change under various conditions such as temperature, seasonal changes and stress. The aim of this study is to, investigate the effect of salinity stress on the composition of rosemary essential oils.

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Table 1: Soil test results of experimental medium

Soil texture	P (mg/kg)	K (mg/kg)	N (%)	pH	EC (dS/m)
Sandy loam	52	40	0.03	7.33	0.60

MATERIALS AND METHODS

The experiments were performed at Lahidjan, Guilan Province, Iran (longitude 50'2″E and latitude 27'15″N). Trials were conducted in triplicate using a randomized complete block design with four treatments. Thirty-six rooted rosemary cuttings were transplanted into pots filled with garden soil. Soil analysis results are shown in Table 1. Salinity stress was applied a month after transplantation. Four saline water samples were used as treatment: tap water (S_1), NaCl 100 mM solution (S_2), NaCl 150 mM solution (S_3) and water from the Caspian Sea (S_4). The salinity of the water from the Caspian Sea was 11-13 g/l. Pot irrigation was carried out three times a week. Weeds were removed mechanically and no pesticides or nutrition sprays were applied during the growing season. The leaves were dried naturally in suitable dry and dark conditions. Twenty grams of the air-dried leaves was placed in a round-bottom flask of the Clevenger apparatus and distilled water was added. The essential oils of the air-dried rosemary were then obtained by hydrodistillation for 3–3.5 h. GC/MS analysis was performed with Agilent GC8690N model, capillary column (30m×0.25mm; 0.25µm film thickness composed of% phenylmethylpolysiloxane). Helium (He) was used as the carrier gas at a flow rate of 1.0 ml/min. The split mode had a ratio of 1:20 and the injection port was set at 250°C. The type and concentration of each component of the essential oils were determined by chromatography. Statistical data were analysed using SPSS software and diagrams were drawn in Excel.

RESULTS

Salinity was shown to affect the composition of rosemary essential oils with a significant probability of 1% (Table 2). Comparison of the means showed that the highest percentage of essential oils (95%) was obtained from plants subjected to S_4 treatment (Caspian Sea water) and the lowest percentage (4%) was obtained from plants subjected to S_1 treatment (water) (Fig. 1). Study of mass spectra identified 30 compounds in the essential oils of plants subjected to S_1 (water) treatment, 22 compounds in the oils of those subjected to S_2 treatment (salt water, NaCl 100 mM), 25 compounds in the oils of those subjected to S_3 treatment (salt water, NaCl 150 mM) and 26 compounds in the oils of those subjected to S_4 treatment (Caspian Sea water) (Table 3). The most important constituents of the essential oils were 1,8-cineole (27.95%–49.14%), borneol (15%–17.81%) and camphor (10.24%–14.42%).

Table 2: Analysis of variance for effect of salinity different levels on essential oil of Rosemary

Source	df	Sum of Squares	Mean Square	F
Replication	2	0.002	0.001	2.600 *
Treatments	3	0.48	0.160	549.05 **
Error	6	0.002	0.000	
CV (%)			0.001	

*and **: Significant at the 5% and 1% of probability levels, respectively. ns: Non- significant.

Table 3: Essential oil compounds of rosemary shoots identified under different levels of salinity

Compounds	S_1 (Tap water)	S_2 (NaCl 100 mM)	S_3 (NaCl 150 mM)	S_4(Caspian Sea water)
1,8 – Cineol	42.33	34.97	49.14	27.95
Borneol	17.05	15.13	15	17.81
Camphor	11.51	8.44	10.24	14.42
β-Pinene	4.21	2.03	4.18	3.89
α –Pinene	3.64	1.41	4.06	3.62
δ-2-Carene	1.83	1.33	1.64	2.96
Terpine -4-ol	1.50	1.78	1.46	1.32
Cryptone	1.47	-	1.04	-
Camphone	1.46	0.65	1.96	1.97
α –Terpineol	1.38	2.30	0.96	0.78
Caryophylene oxide	1.06	1.47	0.75	2.20
Bornyl format	1.05	1.88	1.36	1.64
β-Caryophylene	1.03	0.97	0.31	0.72
Cuminal	1.01	-	1.09	-
Myrtenol	0.96	0.59	0.74	1.72
Sabinene hydrate	0.78	0.65	-	0.61
α – Cadinol	0.77	0.90	0.61	2.34
Terpinolene	0.58	-	0.60	-
α – Campholene aldehyde	0.52	0.70	-	1.11
Pinocarvone	0.52	0.49	0.59	0.71
Linalool	0.35	-	-	-
α –Terpinene	0.36	0.83	0.45	0.95
1- Terpineol	0.32	-	-	0.78
α -Phellandrene	0.31	-	0.32	-
α - Terpinolene	0.28	-	0.60	-
α - Thujene	0.27	-	0.42	-
Gurjunene	0.24	-	-	-
Bornyl acetat	-	-	-	0.57

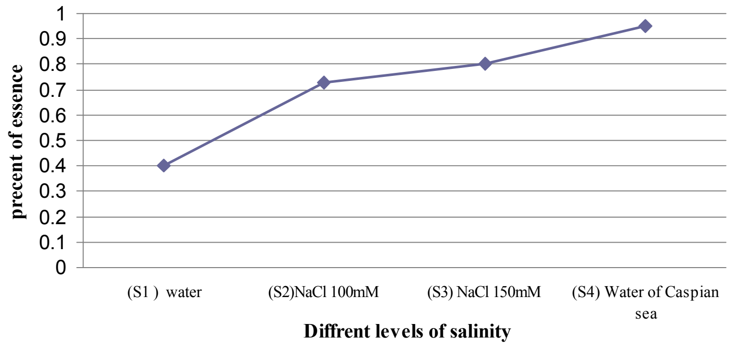

Fig. 1: Effect of salinity different levels on essential oil of Rosemary

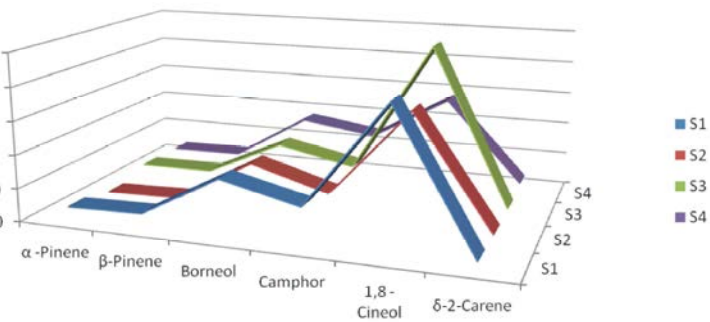

Fig. 2: Effect different salinity stress on percent of essential oil compounds Rosemary
S_1 (tap water), S_2 (NaCl 100 mM solution), S_3 (NaCl 150 mM solution) and S_4 (the Caspian Sea water)

The results of qualitative analysis of rosemary essential oils at different levels of salinity showed that 1,8-cineole was the most important combination of essential oils (Fig. 2), followed by borneol (15%–17.81%) and then camphor (10.24%–14.42%) (Table 3). The results in Table 3 indicated that some compounds such as Gurjenun and Linalool in salinity conditions are at least S_1 and S_4 completely remove in water of Caspian Sea and instead of other compounds such as Bornyl acetate is produced in the plant.

DISCUSSION

The concentration of the constituents of essential oils changes depending on climate, geographical and seasonal variation and stress. Emadi and Yassa [2] suggested that the constituents of essential oils vary with different seasons. Miguel *et al*. [10] studied the effects of harvest date, growth substrate and fertilizers on the composition of essential oils and found that the chemical composition was very sensitive to temperature, photoperiod and harvest time but was less affected by growth substrate and fertilizers. According Dow *et al*. [11], salinity reduces the yield of essential oils in plants belonging to the Lamiaceae family, possibly because of the limited supply of calcium (Ca) from the roots to the branches and alterations in the ratio of Ca to abscisic acid (ABA) in the leaves. El-Keltawi *et al*. [12] examined the effect of water salinity on marjoram and other mint species and found that salinity reduced the yield of essential oils by 20%. Salinity stress has also been shown to reduce the amount of essential oils in slice devile, fennel and ajowan.

In the present study, water from the Caspian Sea was used for the S_4 treatment. The salinity of the Caspian Sea water was first studied in 1897 by Lybdntsf, who measured the salinity of samples of dried sea salts and identified an archived chlorine ratio of 2.38. The Caspian Sea is one of the primary sources of water in Iran that moving in the desert can be used to grow many plants of the halophyte. The average salinity of the Caspian Sea is 12.85 g/l [6]. The amount of free Ca in ocean water is 400 ppm and the ratio relative to chloride ions is 0.021. Thus, the ratio of Ca to chloride ions is slightly greater in ocean water than in the Caspian Sea [13]. The different constituents of rosemary essential oils have different applications. Camphor is a disinfectant that is also used in the manufacture of rubber, paper, perfume and cosmetics; in saponification reactions; industrial adhesives; lubricants; blending resins; solvents; plastics and paint. 1,8-Cineole is widely used in the preparation of pharmaceuticals. It is also used as a local anaesthetic and disinfectant in the treatment of inflammation and for inducing sputum and lowering blood pressure [14]. Furthermore, it is used in domestic sprays and cleaning products; in the manufacture of skin and hair oils, perfumes and deodorants and as an insect repellent. Limonene is the main constituent of citrus essential oils. It has toxic and stimulating effects on the skin and its vapours neutralize meningococcal disease-causing germs within 15 min, typhoid-causing bacilli in at least an hour, pneumococcal infection-causing bacteria in 1–3 h, *Staphylococcus aureus* in 5 min, *Streptococcus* spp. in 3–12 h and diphtheria bacilli in 20 min. It is also used in the formulation of medicinal products such as sodium bicarbonate tablets and anti-septic ointments and in the manufacture of vitamin A [6].

α-Pinene is used in the manufacture of perfumes and insecticides and camphene is used in the manufacture of perfume. Therefore, the importance of plants rich in essential oils is thus evident [14]. According to study, 31 of the 52 compounds identified in rosemary essential oils occur in multiple seasons. Compounds other than α-pinene, limonene, bornyl acetate and 1,8-cineole are unaffected by seasonal change [15]. In another study, rosemary oil was investigated in 14 plant populations in Tunisia and in the ecoregion. 1,8 - cineol was variable 20.34 percent to 45.79 percent of the population [16]. Many studies recommended the use of medicinal plants, especially rosemary, because of its beneficial constituents.

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