Determination of Volatile Oil Constituents in Root and Shoot of *Moringa peregrina* (Forssk.) Fiori in Saline Soil

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Received: 15 Feb. 2014 **Accepted:** 27 April 2014

Abstract

Background: ***Moringa peregrina*** **(Forssk.) Fiori. is a small tree, which grows in south east of Iran. The shoot and root of *M. peregrina* contain volatile oil which has several isothiocyanates.**

Objective: **To study the effect of salinity on volatile constituents of root and shoot of *M. peregrina*.**

Methods: **The experiment was conducted on randomized complete blocks design (RCBD) with 8 treatments and 3 replications. The treatments were consist of control, 2, 4, 6, 8, 10, 12 and 14 dS/m. The volatile constituents were determined by GC and GC/MS.**

Results: **The results showed that the main constituent of volatile oil in control treatment was 1,2–benzendicarboxylic acid, bis (2-methyl propyl) (29.02%) which decreased with increasing salinity. In salinity treatments, the isothiocyanates compounds such as isobutylisothiocyanate and 2-isothiocyanatepropan were increased. In control level of salinity, the main compound of root volatile oil was thiocyanic acid phenylmethyl ester (29.6%) but was not found in the salinity treatment. The content of isothiocyanates in the shoot increased with increasing salinity up to 4dS/m.**

Conclusion: **The level of salinity had significant effect on volatile oil content and component of *M. peregrina*.**

Keywords: ***Moringa peregrina,*** **Moringaceae, Salinity, Volatile oil**

Introduction

Moringa peregrina (Forssk.) Fiori is a desert tree of the monogeneric family Moringaceae, distributed from tropical Africa to east India [1, 2]. Moringa tree is growing in Sistan and Baluchestan, the south east province of Iran. This tree has often numerous stems with raised grey branches [1].

M. peregrina is cultivated as a source of gums, oil and pungent principals [3]. Occurrence of several isothiocyanates in the volatile oil of the seed kernel and leaves of *M. peregrina* has been reported previously [4].

Isothiocyanates (hydrolyzed products of glucosinolates) have various biological activities including antioxidative, antibacterial, anticancer and chemoprotective properties [5, 6]. In several studies, it has been shown that there is an inverse correlation between dietary intake of isothiocyanates and cancer risk of several organs. These findings have created more interest about isothiocyanates as potential cancer-preventive agents in humans [7].

The salinity affects primary carbon metabolism, plant growth and development by ion toxicity, induced nutritional deficiency, water deficits and oxidative stress. Moreover, it modulates the levels of secondary metabolites, which are physiologically important particularly in stress tolerance [8, 9].

Secondary metabolites, phenolic compounds in particular, are involved in plant response to biotic and abiotic stresses and provide a significant contribution to the antioxidant activity of plant tissues [10]. Also, enhanced synthesis of determined secondary metabolites under stressful conditions is believed to protect the cellular structures from oxidative damage [11].

Although *Moringa peregrina* (Forssk.) Fiori is one of the medicinal plants with several isothiocyanates compounds in volatile oil and its cultivation is continuously being extended in the world including Iran, no information is available about the effect of salinity on its volatile oil constituents. The present study was carried out to explore the effects of salinity on *Moringa* volatile oil constituents.

Materials and Methods

Plant Material

Seeds of *M. peregrina* were obtained from the department of Natural Resources of Chabahar, Sistan and Baluchestan province, Iran, and identified by the Institute of Medicinal Plants, ACECR. This study has been conducted in the Institute of Medicinal Plants-ACECR, Iran, on the base of randomized complete blocks design (RCBD) with 8 treatments and 3 replications. Ten seeds were sown in every pot which was filled with sand and exposed to salinity (natural saline water) included 8 levels of salinity (control, 2 dS/m, 4dS/m, 6 dS/m, 8 dS/m, 10 dS/m, 12 dS/m and 14 dS/m). The pots were irrigated with related saline levels. Natural saline water was obtained from Howz-e-Soltan Lake in Qom, Iran. The major ions of saline water were:128 g/l Na^+, 218.7 g/l Cl^-, 1.23 g/l K^+, 19.5 g/l Mg^{2+}, 0.086 g/l Ca^{2+} and 48.8 g/l SO_4^{2}. The plants were irrigated for 3 months. At the 90^{th} day, the plants were transferred to lab for next measurements.

Phytochemical Evaluations of Volatile Constituents

The powdered of air dried root and shoot (40 g of each) were mixed with distilled water (400 ml), separately. The mixtures were left for autolysis at 25°C for 17h in a container. Autolysis which is hydrolytic breakdown of glucosinolates led to the formation of volatile isothiocyanates by separating the glucose moiety from glucosinolate. The mixture was submitted for 3h water-distillation using an Clevenger apparatus. Volatile constituents were collected in the organic layer (cyclohexane) and stored at +4°C until the moment of analysis.

GC Analysis

A Younglin Acm6000 GC instrument using capillary column (30m × 0.25mm; film thickness: 0.25 µm) was used for analysis of volatile constituents and DCM extracts. The carrier gas was helium with a flow rate of 0.8ml/min. The oven temperature was programmed from 50°C to 300°C at 15°C/min. Injector and detector temperatures were set at 290°C.

GC/MS Analysis

Determination of volatile constituents was performed on an Agilent 5973 A with Mass detector under the following conditions: injection volume, 0.1µl, HP-5 MS capillary column (30 m × 0.25mm; film thickness: 0.25 µm); carrier gas, He; flow rate, 0.8 ml/min; injector temperature, 290°C; temperature program, 50-300°C at 15°C/min; mass spectra: electronic impact, ionization potential 70 eV, ion source temperature 220°C, ionization current 1000µA, resolution 1000 and mass range 30-300. Identification of the constituents was performed by computer matching against the library spectra (library database Wiley 275), their retention indices with reference to an n-alkane series in a temperature programmed run, interpreting their fragmentation pattern and comparison of the mass spectra with the literature data [13, 14].

Results

The identified volatile constituents and their relative percentages in root and shoot of *M. peregrina* at control level are listed in Table 1. The results indicated that 1, 2–benzendicarboxylic acid, bis (2-methyl propyl) (29.02%) and thiocyanic acid, phenylmethyl ester (29.6%) was the main volatile constituents of shoot and root, respectively.

The volatile constituents and their relative percentages in root and shoot of *M. peregrina* at 2 and 4dS/m salinity levels are listed in Table 2 and 3, respectively. At 2 and 4 dS/m salinity levels, isobutyl isothiocyanate (49.14%) and 2-isothiocyanatepropan (22.45%) were the main volatile constituents in shoot, and also benzyl isothiocyanate, 1, 2-benzenedicarboxylic acid, bis (2-methyl propyl) and hexadecanoic acid were the maximum components in root.

According to tables 4, 5, 6, 7 and 8, the highest percentage of volatile oil constituents in root of *M. peregrina* was benzyl isothiocyanate (23.68%), but n-butylisothiocyanate (39.56%) and 2-isothiocyanatepropan (22.27%) were the main constituents in shoot.

Table 1- Volatile components of root and shoot of *M. peregrina* (Forssk.) Fiori in control treatment

Volatile Components	Ki	Root (%)	Shoot (%)
1,2-benzenedicarboxylic acid, bis (2-methyl propyl)	1861	13.29	29.02
Isobutyl isothiocyanate	946	-	3.16
Benzene,1-(1,5-dimethyl-4-hexenyl)-4-methyl	1478	-	7.44
Spathulenol	1573	-	9.29
Caryophyllene oxide	1579	-	11.01
Dibutyl phthalate	1952	2.38	4.61
Farnesyl Acetone	1911	-	6.05
Thiocyanic acid, phenylmethyl ester	1359	29.6	1.49
Hexadecanoic acid, ethyl ester	1987	11	-

Table 2- Volatile components of root and shoot of *M. peregrina* (Forssk.) Fiori in 2 dS/m salinity level

Volatile Components	Ki	Root (%)	Shoot (%)
1,2-benzenedicarboxylic acid, bis(2-methyl propyl)	1861	25.17	5.78
Isobutyl isothiocyanate	946	1.03	49.14
2-isothiocyanatepropan	818	1.70	22.45
n-butylisothiocyanate	945	1.03	-
Dibutyl phthalate	1952	4.93	1.57
Hexadecanoic acid, ethyl ester	1987	2.39	-
Hexadecanoic acid	1963	25.40	2.40
Benzyl isothiocyanate	1358	25.45	1.59
Benzyl nitrile	1131	1.35	-
Benaldehyde	951	1.21	-

Table 3- Volatile components of root and shoot of *M. peregrina* (Forssk.) Fiori in 4 dS/m salinity level

Volatile Components	Ki	Root (%)	Shoot (%)
1,2-benzenedicarboxylic acid, bis(2-methyl propyl)	1861	25.17	5.78
Isobutyl isothiocyanate	946	1.03	49.14
2-isothiocyanatepropan	818	1.70	22.45
n-butylisothiocyanate	945	1.03	-
Dibutyl phthalate	1952	4.93	-
Hexadecanoic acid, ethyl ester	1987	2.39	-
Hexadecanoic acid	1963	25.40	-
Benzyl isothiocyanate	1358	25.45	-
Benzyl nitrile	1131	1.35	-
Benaldehyde	951	1.21	-

Table 4- Volatile components of root and shoot of *M. peregrina* (Forssk.) Fiori in 6 dS/m salinity level

Volatile Components	Ki	Root (%)	Shoot (%)
1,2-benzenedicarboxylic acid, bis(2-methyl propyl)	1861	5.42	4.92
Benzene,1-(1,5-dimethyl-4-hexenyl)-4-methyl	1478	4.49	1.73
2-isothiocyanatepropan	818	7.7	22.27
n-butylisothiocyanate	945	2.62	39.56
Dibutyl phthalate	1952	1.15	0.083
Caryophyllene oxide	1579	7.12	-
Benzyl isothiocyanate	1358	23.68	2.51
Benzyl nitrile	1131	5.52	-
Benaldehyde	951	9.39	1.04
Neral	1266	13.30	-
Limonene	1020	10.55	-
Spathulenol	1573	5.12	-
Isopropyl isothiocyanate	835	-	4.23

Table 5- Volatile components of root and shoot of *M. peregrina* (Forssk.) Fiori in 8 dS/m salinity level

Volatile Components	Ki	Root (%)	Shoot (%)
1,2-benzenedicarboxylic acid, bis(2-methyl propyl)	1861	5.42	4.92
Benzene,1-(1,5-dimethyl-4-hexenyl)-4-methyl	1478	4.49	-
2-isothiocyanatepropan	818	7.70	22.27
n-butylisothiocyanate	945	2.62	39.56
Dibutyl phthalate	1952	1.15	-
Caryophyllene oxide	1579	7.12	-
Benzyl isothiocyanate	1358	23.68	2.51
Benzyl nitrile	1131	5.52	-
Benaldehyde	951	9.39	-
Neral	1266	13.30	-
Limonene	1020	10.55	2.37
Spathulenol	1573	5.12	-
Isopropyl isothiocyanate	835	-	4.23

Table 6- Volatile components of root and shoot of *M. peregrina* (Forssk.) Fiori in 10 dS/m salinity level

Volatile Components	Ki	Root (%)	Shoot (%)
1,2-benzenedicarboxylic acid, bis(2-methyl propyl)	1861	5.42	4.92
Benzene,1-(1,5-dimethyl-4-hexenyl)-4-methyl	1478	4.49	-
2-isothiocyanatepropan	818	7.7	22.27
n-butylisothiocyanate	945	2.62	39.56
Dibutyl phthalate	1952	1.15	-
Caryophyllene oxide	1579	7.12	-
Benzyl isothiocyanate	1358	23.68	2.51
Benzyl nitrile	1131	5.52	
Benaldehyde	951	9.39	-
Neral	1266	13.30	-
Limonene	1020	10.55	2.37
Spathulenol	1573	5.12	-
Isopropyl isothiocyanate	835	-	4.23

Table 7- Volatile components of root and shoot of *M. peregrina* (Forssk.) Fiori in 12 dS/m salinity level

Volatile Components	Ki	Root (%)	Shoot (%)
1,2-benzenedicarboxylic acid,bis(2-methyl propyl)	1861	5.42	4.92
Benzene,1-(1,5-dimethyl-4-hexenyl)-4-methyl	1478	4.49	-
2-isothiocyanatepropan	818	7.7	22.27
n-butylisothiocyanate	945	2.62	39.56
Dibutyl phthalate	1952	1.15	-
Caryophyllene oxide	1579	7.12	-
Benzyl isothiocyanate	1358	23.68	2.51
Benzyl nitrile	1131	5.52	
Benaldehyde	951	9.39	-
Neral	1266	13.30	-
Limonene	1020	10.55	2.37
Spathulenol	1573	5.12	-
Isopropyl isothiocyanate	835	-	4.23

Table 8- Volatile components of root and shoot of *M. peregrina* (Forssk.) Fiori in 14dS/m salinity level

Volatile Components	Ki	Root (%)	Shoot (%)
1,2-benzenedicarboxylic acid, bis(2-methyl propyl)	1861	5.42	4.92
Benzene,1-(1,5-dimethyl-4-hexenyl)-4-methyl	1478	4.49	-
2-isothiocyanatepropan	818	7.7	22.27
n-butylisothiocyanate	945	2.62	39.56
Dibutyl phthalate	1952	1.15	-
Caryophyllene oxide	1579	7.12	-
Benzyl isothiocyanate	1358	23.68	2.51
Benzyl nitrile	1131	5.52	-
Benaldehyde	951	9.39	-
Neral	1266	13.30	-
Limonene	1020	10.55	2.37
Spathulenol	1573	5.12	-
Isopropyl isothiocyanate	835	-	4.23

Discussion

The main volatile oil constituents of shoot in control treatment was 1, 2-benzenedicarboxylic acid, bis (2-methyl propy l), but salinity caused changes in the volatile constituents. Therefore, isothiocyanates such as isobuty l isothiocyanate and 2-isothiocyanatepropan at 2 and 4 dS/m salinity levels and n-butylisothiocyanate and 2-isothiocyanatepropan at 6, 8, 10, 12 and 14 dS/m increased.

In control level, the main component volatile oil of root was thiocyanic acid, phenylmethyl ester but these components altered with increasing salinity. At 2 and 4dS/m salinity level, hexadecanoic acid, benzyl isothiocyanate and 1, 2-benzenedicarboxylic acid, bis (2-methyl propyl) were the main components, but in higher levels of salinity (6, 8, 10, 12 and 14 dS/m), only benzy l isothiocyanate was the main volatile oil constituent. The results showed that there are more amounts of isothiocyanate compounds in *Moringa peregrine* root than shoot in control conditions (Figures 1 and 2). Furthermore, the salinity led to producing more amounts of isothiocyanates in volatile oil constituents of shoot (Figure 1). The results showed that shoot isothiocyanates increased with salinity up to 4dS/m. An increase of volatile oil yield by salinity has been reported earlier in other plant species, e.g. saga (*Salvia officinalis* L.) [15] and *Mentha pulegium* [16]. The stimulation of essential oil production under a moderate degree of salinity could be due to a higher oil gland density [17]. Salt stress may also affect the essential oil accumulation indirectly through its effect on either net assimilation or the partitioning of assimilates among growth and differentiation processes [17].

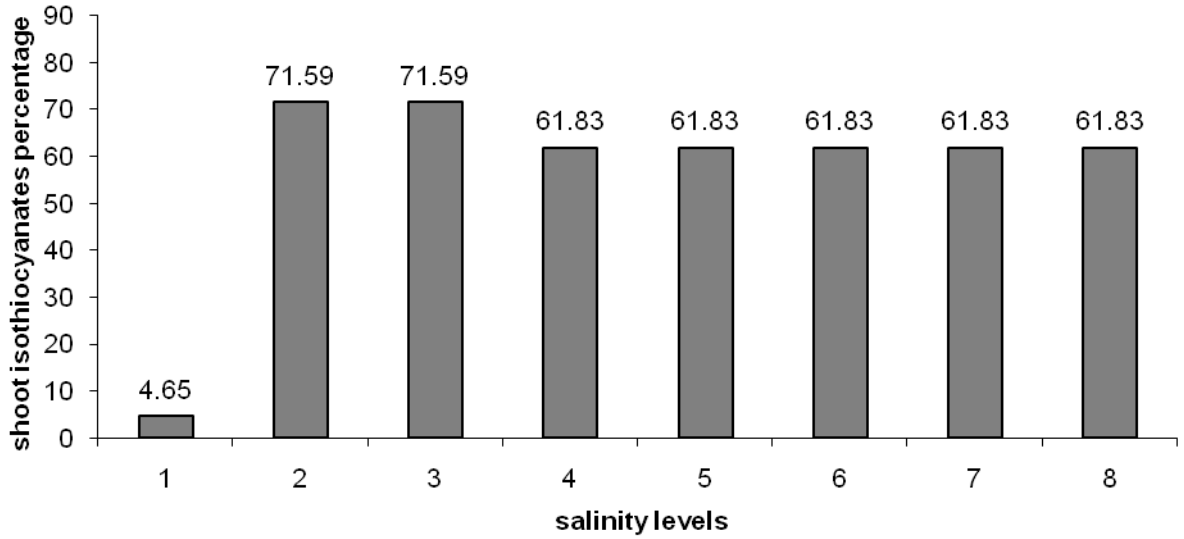

Figure 1- The effect of salinity levels on shoot isothiocyanates percentage

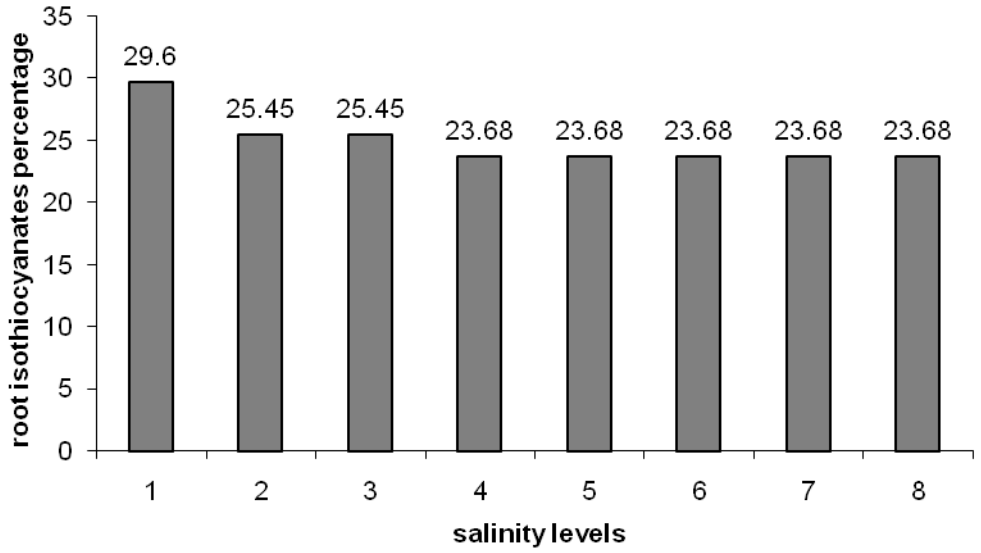

Figure 2- The effect of salinity levels on root isothiocyanates percentage

Glucosinolates, a class of secondary metabolites, are nitrogen and sulfur containing compounds mainly found in Capparales and almost exclusively in Brassicaceae, which include *Brassica* crops of economic and nutritional importance, as well as the model plant, *Arabidopsis thaliana* [18, 19]. Glucosinolates are biosynthesized from tryptophan and seven other protein amino acids including methionine and phenylalanine [20]. It has been suggested that high concentrations of organic solutes in the cytoplasm, including proline, sucrose, glycinebetaine and secondary metabolites, such as glucosinolates, contributes to the osmotic balance [21].

Conclusion

Although the isothiocyanates amounts of shoot increased in saline condition, but their amounts in root decreased. The results showed that shoot isothiocyanates increased with salinity up to 4dS/m level and decreased in 6 dS/m. Moreover no more reduction in isothiocyanates amounts was observed in higher levels of salinity such as 8, 10, 12 and 14 dS/m. In other words the isothiocyanates amount remained constant and did not decrease more with increasing salinity. Since isothiocyanate amounts in shoots are more than roots in all studied salinity levels and according to isothiocyanates importance, it can be suggested to use salinity up to 4 d/Sm to increase isothiocyanates in *Moringa peregrina* shoot.

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