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Full Length Research Paper

Comparative Analysis of Habitat Effects on Phytochemical Constituents in Miswak (*Salvadora persica* L.)

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***Salvadora persica* is a popular medicinal plant in the Middle East countries. It is well adapted to a wide range of habitats. In this study we have compared the effect of two different habitats (Al-ahsabah valley** and **Shada Mountain) in Al-Baha City, south west of Saudi Arabia on the content of some primary and secondary metabolites in *S. persica*. The results show that soil texture, soil moisture and organic matter contents varied in the studied regions. This variation was accompanied by differences in total count and distribution of Mycorrhizal spores in both studied habitats. Soil total count of mycorrhizal spores and root colonization percentage in Shada Mountain was significantly higher than that in Al-Ahsabah valley. Moisture content, carbohydrates, proteins and amino acids concentrations in leaves and roots of plants collected from Al-ahsabah valley were significantly higher than those collected from Shada Mountain. Gas chromatography mass spectrometry (GC-MS) analysis showed that benzene, (isothiocyanatomethyl) was the most abundant analyt in both extracts. There was a slight variation in the secondary metabolites pattern, where benzene, 1-isocyano-2-methyl- in extract of *S. persica* roots collected from Al-Ahsabah valley substituted with Benzyl nitrile in *S. persica* roots of Shada Mountain. Taken together we could conclude that, different habitats in the studied regions affect markedly the concentration of its primary metabolites and to less extent the secondary metabolites. Isolation of the active phytochemical constituents from plants of different habitats and studding of its biological activity will definitely give fruitful results.**

Key words: Miswak, soil factors, mycorrhiza, carbohydrates, protein, amino acids, gas chromatography mass spectrometry (GC-MS).

INTRODUCTION

Salvadora persica L. (family Salvadoraceae) is an evergreen shrub, 4 to 6 m tall with a short trunk, white bark, fleshy leaves and drupes, 3 mm diameter smooth fruits (Sher et al., 2010; Wasimuzzama et al., 2010). It is

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commonly called Arak, Miswak or toothbrush tree where its roots and branches are used to prepare chewing sticks in many third world countries (Elvin-Lewis, 1982; Almas and Al-Lafi, 1995). The tree is well adapted to different edaphic and topographical factors and widespread in desert areas of Africa and South Asia. It has been found growing both in the plains and the hills. It could be found in dry water courses and rocky depressions; however, it occurs more widely in wet sites including riverbanks, perimeters of waterholes, and drainage lines in arid zones where the ground water is near the surface (Iyenger et al., 1992; Khafagi et al., 2006). Therefore, it can tolerate high rainfall, low humidity, extreme drought, and temperature range from - 3 to +48°C (Iyengar et al., 1992). It thrives on a variety of soils including sandy loam, clayey loam, gravelly, shallow, calcareous and sand dunes; tolerates a degree of salinity or alkalinity with pH of 6.5 to 8.5. Thus, it is distributed in many countries in the Middle East (Al-Samh and Al-Nazhan, 1997; Zodape and Indusekhar, 1997).

Distribution behavior of *S. persica* varies, to some extent, in different countries probably due to the changes in water resources, climatic and edaphic factors and anthropogenic demands (Hassan et al., 2010). It is distributed in India, Sri Lanka, Egypt, Pakistan, Sudan, Ethiopia, Senegal and Gulf including Saudi Arabia (Iyenger et al., 1992; Sher et al., 2010). In Saudi Arabia, *S. persica* has wide distribution pattern from 0 to 1500 m altitude in the whole arid and semi-arid ecosystem. It mostly grows on relatively open south facing slopes in Saudi Arabia and has a preference to the moist habitat (Al-Yemeni and Zayed, 1999; Sher et al., 2010).

S. persica is one of the most popular medicinal plants in the Muslim world. Several studies identified a variety of biologically active constituents in *S. persica* extracts. These chemical compounds are classified as volatile oils, flavonoids, alkaloids, steroids, terpenoids, saponins, carbohydrates, vitamins, and salts mostly as chlorides (Rajesh et al., 2009), in addition to organic sulphur compounds (Daxenbichler et al., 1991) and lignin glycosides (Kamel et al., 1992) defined in almost every part of the plant including leaves, roots and stem bark and have a pharmaceutical importance (Kumar et al., 2012).

Many reviews presented data on high number of different phytochemicals like benzylisothio-cyanate, benzylamides, trimethylamine, salvadourea, salvadorine, salvadoricine, rutin quercetin, 1-8-cineole, α-tocopherol, α- lonone, α-caryophellene, β-pinene, and β-sisterol (Attar, 1979; Al-Lafi and Ababneh, 1995; Sofrata et al., 2011). Consequently, the plant shows strong antibacterial (Sofrata et al., 2008), antifungal (Al-Mohaya et al., 2002; Hamza et al*.,* 2006), antiulcer (Sanogo et al., 1999), antiparasitic, and antiviral (Ali et al*.,* 2002) characteristics. It is also utilized in most dental treatments and cleansers (Almas, 2002; Al-Otaibi et al*.,* 2004; Almas et al., 2005; Darmani et al., 2006). The World Health Organization (WHO, 1987) and foreign direct investment (FDI, 2000) have recommended and encouraged the use of Meswak as chewing sticks after examination of its effectiveness as an oral hygiene aid. The *Salvadora* species have other medicinal applications in which, all plant parts could be used (Almas et al., 2005; Darmani et al*.,* 2006). The roots are used for chest diseases while the latex is used for treating sores, leaves, root bark, fruits and seeds are used for cough, fever and asthma treatment and as purgative (Mahar and Malik, 2001; Savithramma et al., 2007). Recently, Farag et al. (2017) provided a complete profile of volatiles, sugars, and organic acids in *S. persica* organs.

In addition to its great medicinal value, *S. persica* holds several other potentialities. The tree is suitable for growth in the shelter belts and as wind breaks. The fruits are used as food and the fresh leaves, rich in minerals, are eaten as salad (Sujata, 2015). Leaves also make a good fodder where it is readily consumed by goats and cattle (Abou-Zaid et al., 2015). Resin used during manufacturing stains. Oils of *S. persica*seeds are used for soap and detergent industries (Kumar et al., 2012).

As mentioned previously, *S. persica* is well adapted to different natural habitats. In spite of the tremendous studies that are available regarding the importance of *S. persica* and its bioactive compounds, nothing in the literature, as far as we know, is available to compare the effect of different habitats on its chemical constituents and consequently, its medical value. In the present investigation, a comparative study was conducted to explore the effect of two different habitats (Al-Ahsabah valley and Shada Mountain) in Al-Baha City, south west of Saudi Arabia on the concentrations of some primary and secondary metabolites in *S. persica* extracts*.*

MATERIALS AND METHODS

Collection of soil and plant samples

Soil samples and *S. persica* plants were collected from Al-Ahsabah valley and Shada Mountain, Al-Baha City, south west of Saudi Arabia at latitude of 19° 20' N and longitude 41° 42' E. Soil samples were used for characterization of soil physiochemical properties and isolation and identification of Mycorrhizal spores. The plants were gently washed with distilled water, dried between two paper towels and brought into the laboratory. Freshly collected plants were used for the determination of moisture and organic matter contents in plants leaves and roots in addition to arbuscular mycorrhizal fungi studies in plant roots. Other plants were separated into leaves and roots, shade dried and then ground separately with the help of electronic grinder to fine powder. Water extracts of the powdered tissues were used for primary metabolites estimation. Some secondary metabolites in crude root extract were analyzed using GC-mass spectroscopy.

Characterization of soil physiochemical properties

Soil texture was determined according to the method of Al Yamani

Table 1. Soil texture of Al-Ahsabah valley and Shada Mountain.

Site	Coarse sand (%)	Fine sand (%)	Silt (%)	Clay (%)
Al-ahsabah valley	37.69 ± 0.53[b]	13.83 ± 0.48[b]	37.16 ± 0.89[a]	11.31 ± 1.90[a]
Shada mountain	72.32 ± 1.79[a]	18.28 ± 1.13[a]	6.20 ± 1.38[b]	3.20 ± 0.46[b]

and Al-Desoki (2006). 100 g of soil samples were weighed and sieved with variable sizes sieves (mesh sizes 0.5 to 0.005 mm). Soil particle size was determined depending on the United States Department of Agriculture classification as follows: clay<0.002 mm, silt<0.05 mm, fine sand<0.25 mm and coarse sand<0.5 mm. The weighed soil samples were put separately into the top sieve, and the sieves were shaken for about 1 h by a sieve shaker. After 1 h, each sieve was individually weighed and the relative percentage of clay, silt, fine sand and coarse sand was calculated.

Moisture and organic matter percentages in soil and plant samples were determined using procedures previously described by other investigators (Wilde et al., 1972; Yousef, 1999; Conklin, 2005).

The following formula was used to determine the percentage of organic matter in the soil and plant samples:

$$\left(\frac{\text{Dry sample (g) - incinerated sample (g)}}{\text{Dry sample (g)}}\right) \times 100$$

The percentage of water in the soil and plant samples was determined using the following formula:

$$\left(\frac{\text{Wet sample (g) - Dry sample (g)}}{\text{Wet sample (g)}}\right) \times 100$$

Isolation and identification of mycorrhizal spores in soil samples

The method described by Brundrett et al. (1996) was modified and used to isolate the mycorrhizal spores. Approximately, 50 g of soil were placed into a container and mixed with 1 L of water to obtain a suspension, which was sieved using sieve sizes of 250, 100 and 50 µm. The sieved soil was then rinsed with water several times until clear water seeped through the 50 µm sieve. The sieved water was collected and centrifuged twice. Spores were isolated from denser soil components by carefully disposing of the supernatant and floating debris. Then, the sample was re-suspended in 1.17 M of sucrose until a volume of 30 mL was reached. Next, the suspension was mixed again and centrifuged at 2000 RPM for 10 min. The resulting materials were further sieved by utilizing a 50 µm-sized sieve, and trapped spores were rinsed with water and filtered through a filter paper prior to their transfer to a Petri dish.

Based on morphological characteristics, spores were stained with a mixture of Melzer's reagent and polyvinyl alcohol-lactoglycerol (Koske and Tessier, 1983; Morton and Benny, 1990). The spores were immobilized with a cover slip and examined under a stereomicroscope (CX 41 RF, Olympus Corporation, Philippines) in order to classify them by genus level (Morton and Benny, 1990).

Estimation of colonization and spore density in plant roots

Roots were removed from the lactoglycerol solution and placed on a Petri dish. A piece of paper with grid sizes of 1 cm × 1 cm was placed under the Petri dish. The number of arbuscular mycorrhizal fungi (AMF) was counted under a microscope. The estimated density of AMF was then calculated according to the following equation (Brundrett et al., 1996):

$$\left(\frac{\text{Number of cells with AMF present}}{\text{Total cells}}\right) \times 100$$

Analysis of primary metabolites

The anthrone sulphuric acid method (Fales, 1951; Schlegel, 1956) was used for soluble carbohydrates determination in plant leaves and roots. Soluble proteins content was determined in the plant extracts using Folin reagent as followed by Lowry et al. (1951). The free amino acids were determined depending on the method of Moore and Stein (1948) in the same water extract of proteins and carbohydrates.

GC-mass spectroscopy analysis of secondary metabolites

Crude roots extracts of *S. Persica* were analyzed by GC-Mass spectroscopy (GC/MS (7890A/5975B)) and column (GD-5MS) in the Analytical Chemistry Unit (ACAL), Faculty of Science, Assiut University, Assiut, Egypt. The results were used to determine the analyst composition and their percentage in the samples.

Statistical analyses

All data obtained were subjected to a one-way analysis of variance (ANOVA), using the SPSS statistical package. For comparison of the means, the Duncan's multiple range tests ($p< 0.05$) were used.

RESULTS

The results in Table 1 shows considerable differences in soil texture between the two studied regions. Al-ahsabah valley soil consisted mainly of coarse sand and silt in comparable values (around 37%). However, coarse sand represented the most dominant soil fraction (72.32%) in Shada Mountain. The percentages of silt and clay soil in Al-Ahsabah valley were significantly higher than those in Shada Mountain, while the percentages of coarse and fine sands in Shada Mountain were significantly higher than those in Al-Ahsabah valley.

There were differences also recorded in soil chemical prosperities (Table 2). Al-Ahsabah valley soil had significantly higher soil moisture and organic matter

Table 2. Soil chemical properties of Al-Ahsabah valley and Shada Mountain.

Site	Soil moisture (%)	Soil organic matter (%)	Soil pH
Al-ahsabah valley	47.36 ± 1.79[a]	48.72 ± 0.49[a]	7.84 ± 0.07[a]
Shada mountain	39.20 ± 0.91[b]	36.24 ± 1.05[b]	7.35 ± 0.06[b]

Table 3. Distribution of some arbuscular mycorrhizal fungi as affected by the studied habitats.

Site	Spore count (per 100 g dry soil)	Root colonization (%)	Most dominant spp.
Al-Ahsabah valley	2733.33 ± 305.51[b]	65.67 ± 3.21[b]	*G. fasiculatum* *G. mosseae* *G. macrocarpus* *S. clavispora*
Shada mountain	3066.67 ± 152.75[a]	78.67 ± 3.51[a]	*G. fasiculatum* *G. mosseae* *G. macrocarpus*

contents and pH value compared to Shada Mountain.

As presented in Table 3, soil total count of mycorrhizal spores in Shada Mountain (3066.67 spore/100 g dry soil) was significantly higher than that in Al-Ahsabah valley (2733.33 spore/100 g dry soil). The spores found in the soil samples of both regions belonged to families Glomaceae and Acaulosporaceae. The spores of four mycorrhizal species could be identified in Al-Ahsabah valley namely, *Glomus fasiculatum*, *Glomus mosseae, Glomus macrocarpusand* and *Sclarocystis clavispora*. In Shada mountain, the spores of only three mycorrhizal species were recorded*; Glomus fasiculatum*, *Glomus mosseae* and *Glomus macrocarpus* (Table 3). In addition, it was observed that, the percentage of roots mycorrhizal colonization in Shada Mountain (78.67%) was significantly higher than that in Al-Ahsabah valley (65.67 %). The identified mycorrhizal spores is Figure 1.

Moisture and organic matter percentages in leaves and roots of *S. persica* collected from the studied regions were estimated and presented in Figure 2. Moisture percentage in plant leaves collected from Al-ahsabah valley was significantly higher than that collected from Shada Mountain (Figure 2a). Similar trend was observed for root moisture percentage (Figure 2b). However, no significant differences were recorded in organic matter content of *S. persica* leaves and roots collected from both regions (Figure 2).

Different habitats in the studied regions had marked effects on primary metabolites concentrations as shown in Figure 3. Carbohydrates, proteins and amino acids concentrations in leaves of *S. persica* collected from Al-ahsabah valley were significantly higher than those collected from Shada mountain (Figure 3a). *S. persica* roots exhibited similar attitude where carbohydrates, proteins and amino acids concentrations in plants collected from Al-ahsabah valley were significantly higher than those collected from Shada Mountain (Figure 3b). Figure 3 displayed also that, amino acids represented the leaves main constituent while proteins represented the roots main constituent in plants collected from both studied regions.

The effect of the studied habitats on some secondary metabolites composition of *S. persica* roots crude extracts, retention time in minutes, molecular weight and relative peak area of identified compounds were summarized in Table 4. These obtained results are presented in their elution order on GD-5MS column. GC-MS chromatogram is presented in Figure 4. Four compounds were identified in *S. persica* roots (Figures 5 to 8).

In crude extract of *S. persica* roots collected from Al-Ahsabah valley, three compounds were identified, namely (benzene, 1-isocyano-2-methyl-), benzene, isothiocyanatomethyl)-), and (butylated hydroxytoluene) (Table 4). In extract of *S. persica* roots collected from Shada mountain, the same compounds were identified, however Benzene, 1-isocyano-2-methyl- was substituted by Benzyl nitrile. In both extracts, the most abundant compound was benzene, (isothiocyanatomethyl), its peak area was about 99% (Table 4).

DISCUSSION

Meswak is frequently used for brushing teeth in the Middle East, including Saudi Arabia (Ahmad and

Glomus macrocarpus

Glomus macrocarpusGlomus macrocarpus

Glomus mossaea *Glomus mossaea*

Glomus mossaea

Sclerocystis clavispora

Sclerocystis clavisporaSclerocystis clavispora

Glomus fasiculatum

Glomus fasiculatum Glomus fasiculatum

Figure 1. The most dominant mycorrhizal spores identified in both habitats.

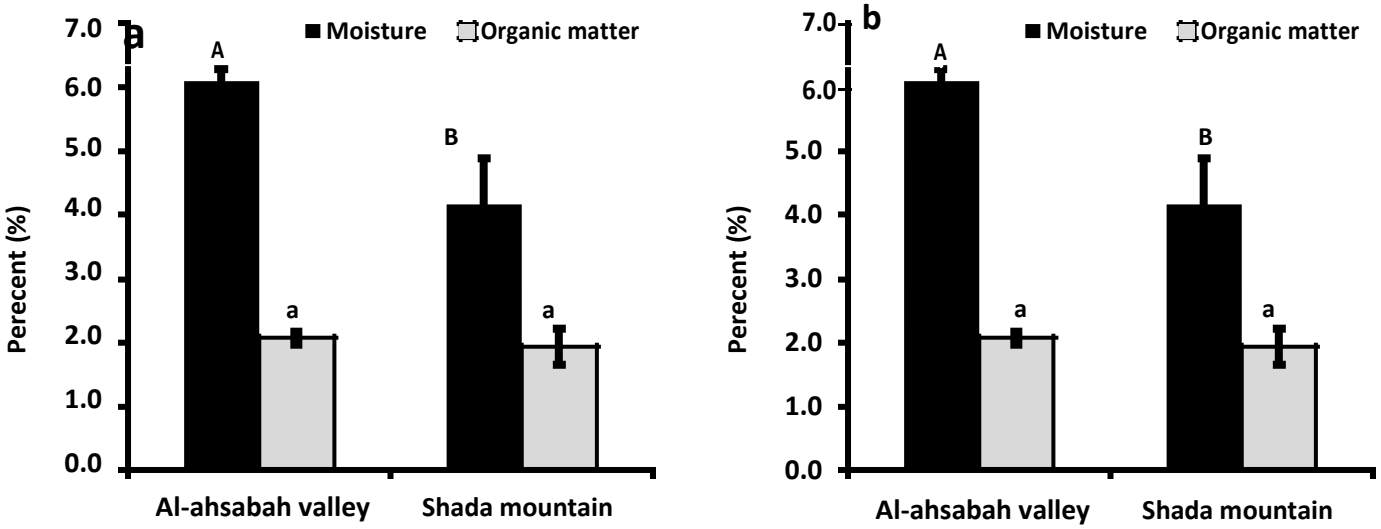

Figure 2. Moisture and organic matter percentage (%) of *S. persica* (I) leaves and (II) roots as affected by the studied habitats. Each point is a mean of three replicates ± SE. The letters A and B represent the statistical significance of each treatment on moisture percentage at P<0.05; a and b similarly represent those of organic matter percentage.

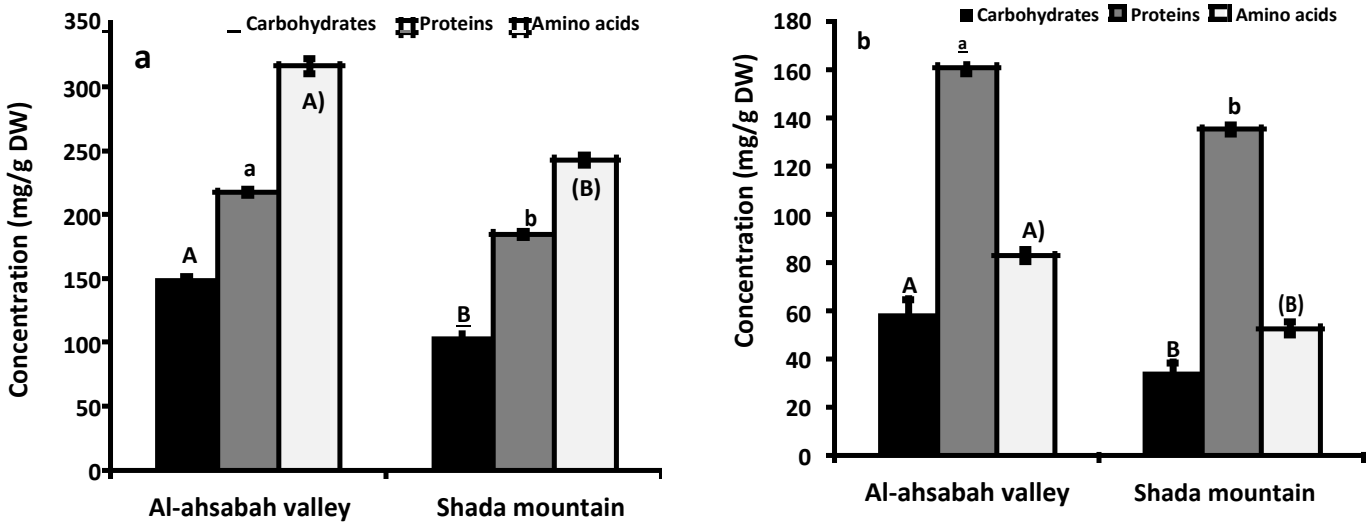

Figure 3. Soluble carbohydrates, proteins and amino acids contents (mg/g DW) of *S. persica*(I) leaves and (II) roots as affected by the studied habitats. The letters A and B represent the statistical significance of each treatment on carbohydrates content at P< 0.05; a and b similarly represent those of proteins content, while (A) and (B) represent those of amino acids content.

Table 4. Some secondary metabolites identified in *S. persica* roots crude extract by GC-MS analysis.

Compound	Retention time (minute)	Molecular weight (g)	Peak area (%)	
			Al-Ahsabah valley	Shada mountain
Benzyl nitrile	12.48	117	-	0.72
Benzene, 1-isocyano-2-methyl-	13.13	117	0.14	-
Benzene, (isothiocyanatomethyl)-	21.72	149	99.79	99.04
Butylated hydroxytoluene	26.82	220	0.07	0.24

Rajagopal, 2014). The plant is also found indifferent habitats including valleys, termite mounds and dunes and at an altitude up to 1800 m (Kumar et al., 2012). The studied regions showed different edaphic factors. Al-ahsabah valley soil consists mainly of coarse sand and silt in comparable values. Coarse sand represents the most dominant soil fraction in Shada Mountain. The percentage of silt and clay soil in Al-ahsabah valley are significantly higher than that in Shada mountain, while coarse and fine sands in Shada mountain are significantly higher than that in Al-ahsabah valley. Al-Ahsabah valley soil has significantly higher soil moisture and organic matter contents compared to Shada Mountain, while both of them showed slightly alkaline soils with pH values 7.83 to 7.53. These results supported by the finding of Kumar et al. (2012), who reported that *S. persica* found on clays, loam, black soils and sand. Sujata (2015) concluded that, *S. persica* is adapted to alkaline, non-saline or very saline soils.

Arbuscular mycorrhizal fungi can strongly influence the metabolism of their host plant (Fontana et al., 2009). These are fungal symbionts that are well known to improve plant nutritional status by enhancing the uptake of essential nutrients and by improving the water supply through an increase in root surface area (Smith and Read, 1997). It also enhances secondary metabolites content (Lu et al., 2015). Both habitats showed variation also in the total count and distribution of arbuscular mycorrhizal fungi. Soil total count of mycorrhizal spores and intensity of root colonization in Shada mountain were significantly higher than those in Al-ahsabah valley. In Al-ahsabah valley four AMR species were defined, while in Shada Mountain only three species were defied. This variation could attribute to the differences in soil moisture and organic matter contents of both regions and could influence the growth and chemical constituents of *S. persica* collected from these regions. Fitter et al. (2000) and Jeffries et al. (2003) concluded that, AMF are found everywhere in land ecosystems and in a variety of climate and soil. Soil pH, mineral constitution and soil

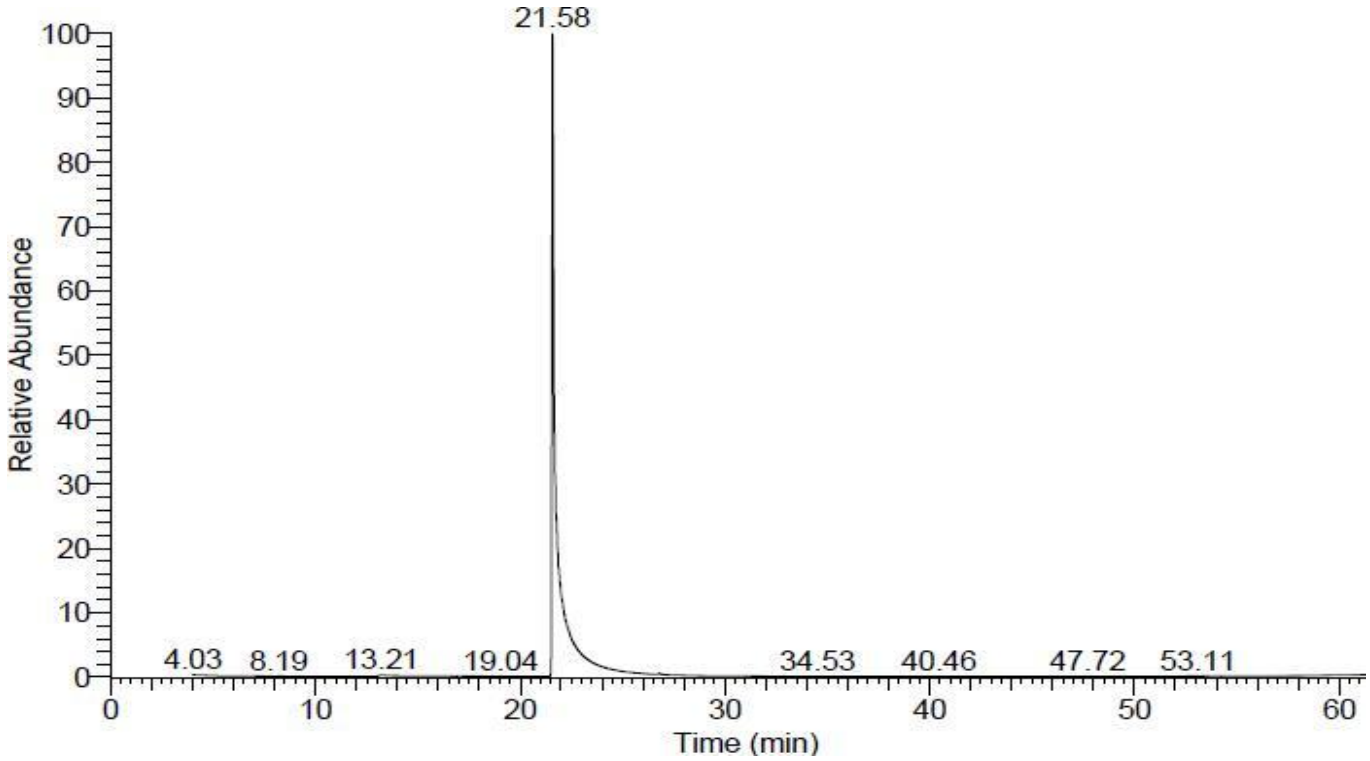

Figure 4. GC-MS chromatogram of *S. persica* root crude extract.

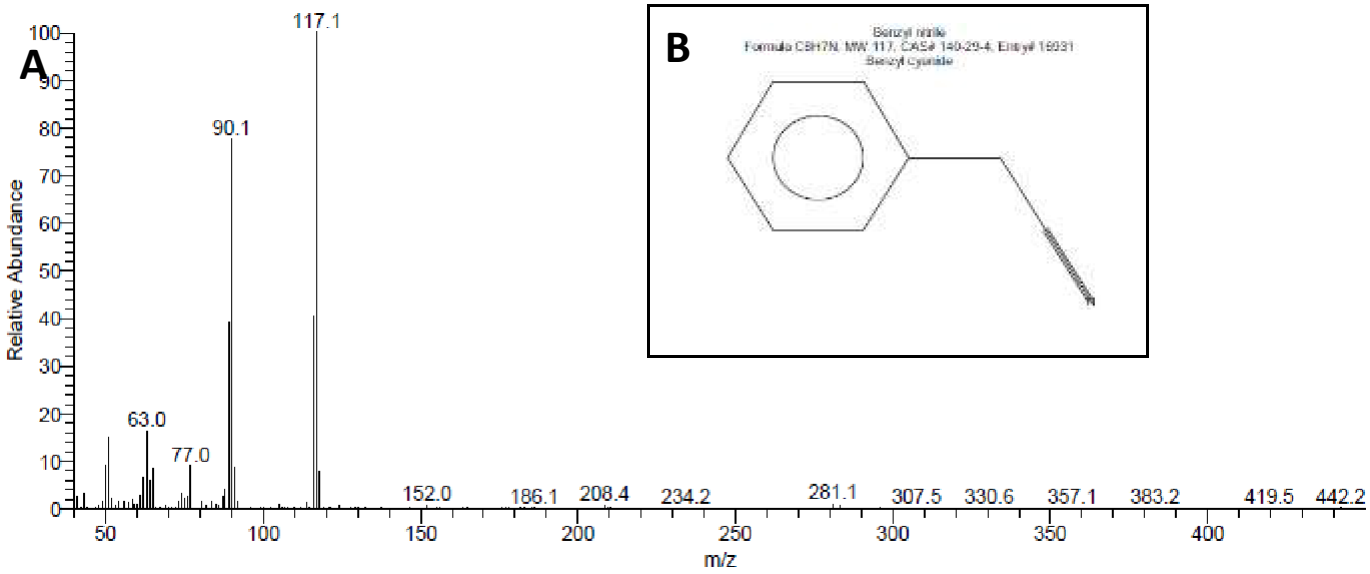

Figure 5. Benzyl nitrile (A) mass spectra, (B) formula.

type has been reported to affect the degree of specialization exhibited by AMF (Klironomos et al.,1993) as well as their growth and colonization (Abler, 2004). Many reports have shown that AMF play an important role in helping plants withstand water deficiency (Augé, 2001; Augé, 2004; Aroca et al., 2008). In general, soil characteristics of such as its texture, pH, presence of microaggregates, main cations and organic matter, can

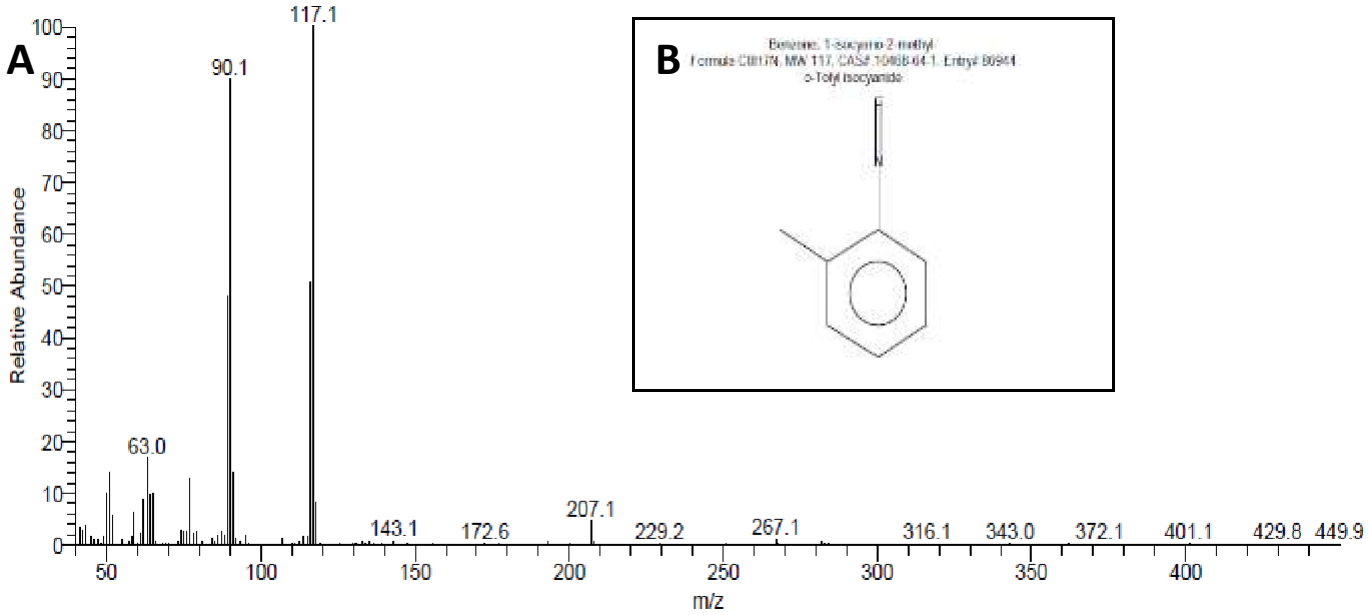

Figure 6. Benzene, 1-isocyano-2-methyl (A) mass spectra, (B) formula.

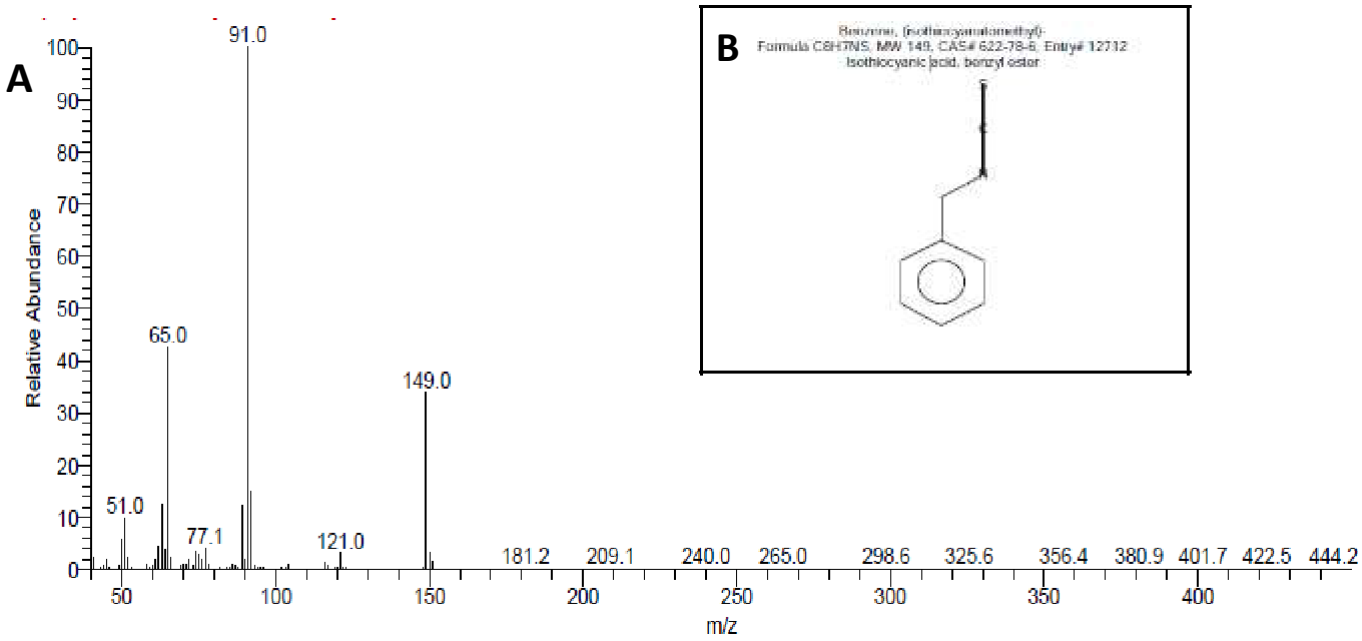

Figure 7. Benzene, (isothiocyanatomethyl) (A) mass spectra, (B) formula.

directly or indirectly influence rhizospheric community (Garbeva et al., 2004).

Different habitats in the studied regions have marked effects on leaves and root moisture content and primary metabolites concentrations. Moisture percentage and carbohydrates, proteins and amino acids concentrations in leaves and roots of plants collected from Al-ahsabah valley in significantly higher than those collected from Shada Mountain. The results of the present study displayed also that leaves body is composed of amino acids while root body is composed mainly of proteins in plants collected from both studied regions. In accordance

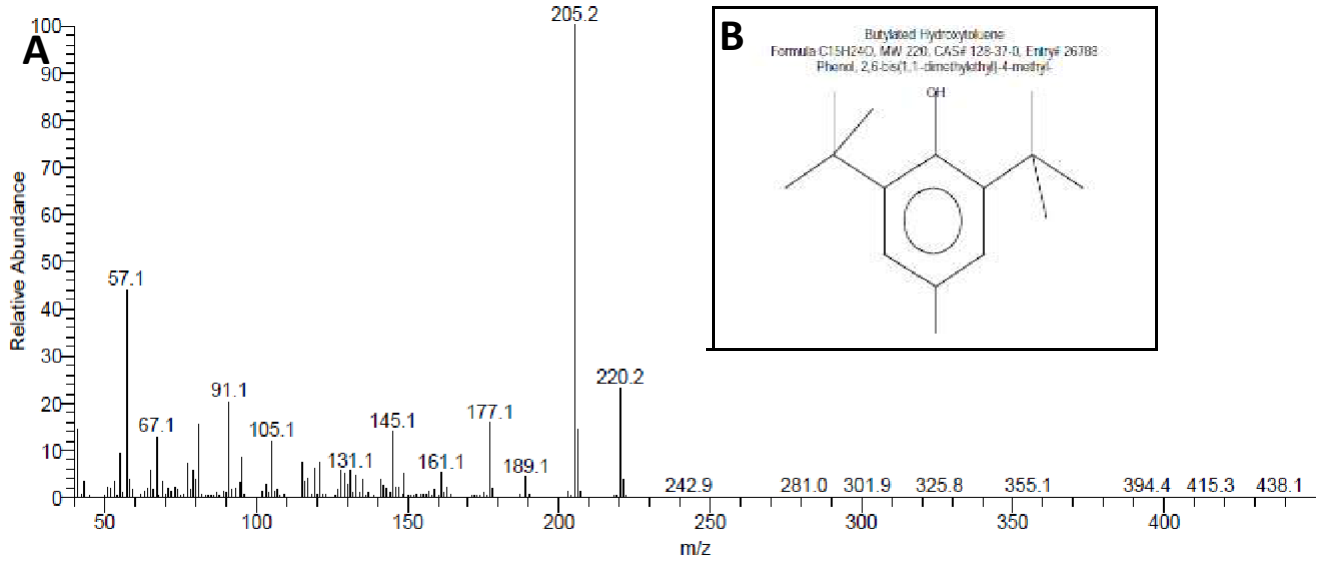

Figure 8. Butylated hydroxytoluene (A) mass spectra, and (B) formula.

with our results, other studies (Bharucha and Rangnekar, 1957; Chaturvedi and Maheshwari, 1980; Joshi et al., 1993) demonstrated that *S. persica* leaves contain high level of different amino acids including glutamic acid, asparagine, valine, aspartic acid, tyrosine, phenylalanine, serine, alanine and threonine.

Traditionally all parts of *S. persica* could be used for many medicinal purposes because of its high content of different secondary metabolites that could be used in the treatment against bacterial and fungal infections and have wide range of health care properties (Arora and Gupta, 2011; Sher et al., 2011; Mohamed and Khan, 2013; Gupta et al., 2015; Kumari and Parida, 2016). In the current study, the studied habitats affect the secondary metabolites composition of *S. persica* roots extract. In crude extract of *S. persica* roots collected from Al-ahsabah valley, Benzene, 1-isocyano-2-methyl- and benzene, (isothiocyanatomethyl), and butylated hydroxytoluene were recorded. In extract of *S. persica* roots collected from Shada mountain, benzyl nitrile was added while benzene, 1-isocyano-2-methyl- disappeared. In both extracts, principal compound was benzene, (isothiocyanatomethyl). benzylisothionate was the main constituent of *S. persica* root extract in many other studies (Christy et al., 2001; Arora and Gupta, 2011).

Benzyl isothiocyanate suggested acting as chemo-preventive agents (Al-Dosari et al., 1992), prevent genotoxic compounds (Attar, 1979), has exhibited broad-spectrum bactericidal activity (Al-Lafi and Ababneh, 1995). Benzylisothio-cyanate and related compound from *S. persica* was reported also to have inhibition of carcinogenic effects of polycylic hydrocarbons (Wasimuzzama et al., 2010), and also shows virucidal activity against Herpes simplex virus-1 (Al-Bagieh, 1992). Butylated Hydroxytoluene is a phenol derivative that is useful for its antioxidant properties (Babu and Wu, 2008; Yehye et al., 2015).

Conclusion

Soil total count of mycorrhizal spores and root colonization percentage in Shada Mountain was significantly higher than that in Al-Ahsabah valley. Moisture content, carbohydrates, proteins and amino acids concentrations in leaves and roots of plants collected from Al-ahsabah valley was significantly higher than that collected from Shada Mountain. Benzene, (isothiocyanatomethyl) was the most abundant analyt in root extract of *S. persica* collected from both regions. Different habitats in the studied regions affect the concentration of its primary metabolites more than secondary compounds. Further study to evaluate the differences in biochemical activity of phytochemicals isolated from plants collected in both regions is needed.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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