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Endophytic fungi associated with endogenous *Boswellia sacra*

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ABSTRACT

El-Nagerabi SAF, Elshafie AE, AlKhanjari SS. 2014. Endophytic fungi associated with endogenous Boswellia sacra*. Biodiversitas 15: 24-30.* Endophytic fungi associated with leaves and stem tissues of *Boswellia sacra* growing in Dhofar Mountains of Oman were investigated from May 2008 through October 2011. The biological diversity, tissue-preference and seasonal variations of fungi were evaluated. Forty-three species and 3 varieties of fungi were recovered as new records from this plant. Of these isolates, 35 species are new reports to the mycoflora of Oman, whereas 12 species were added to the list of fungal flora of the Arabian Peninsula. The genus *Alternaria* (12 species) is the most prevalent genus recovered from 12.5-83.3% of the screened leaves and stem samples, followed by *Aspergillus* (5 species, 3 varieties, 6.9-86.1%), Mycelia sterilia (76.4%), *Rhizopus stolonifer* (62.5%), *Drechslera* (3 species, 40.3-54.2%), *Cladosporium* (3 species, 20.8-52.8%), *Curvularia lunata* (38.8%), *Chaetomium* (2 species, 15.3-26.3%), *Penicillium* spp. (9.8-27.8%), *Fusarium* (9 species, 6.9-27.8%), *Ulocladium consortiale* (27.8%), *Mucor hiemalis* (19.5%), and the remaining species (*Scytalidium thermophilum*, *Phoma solani*, *Taeniolella exilis*, and *Botryodiplodia theobromae*) exhibited very low levels of incidence (4.2-11.1%). Endophytic colonization of the leaf tissues was greater (43 species, 3 varieties) comparable to stem tissues (25 species). This indicates heterogeneity and tissue-preference, with no evidence of seasonal variation. Therefore, the isolation of many fungal species and sterile mycelia supports the biodiversity of the endophytic fungi invading *B. sacra* and the high possibility of isolating more fungal species using advanced molecular techniques

Key words: Dhofar Mountains, endophytes, fungal community, Oman, tissue-preference

INTRODUCTION

Mountain ecosystems are of great interests to the world, covering 24% of the earth surface and supporting 12% of the world population as water source and inhabited by diverse flora and fauna (Anon 2008). In Oman, Dhofar Mountains are distinguished ecosystem with different climatic conditions and diverse vegetation. They have faced rapid development which resulted in noticeable climatic changes and vegetation deterioration. These changes are affecting the flora, fauna and microorganisms including fungi and bacteria which survive on higher plants (Carlile et al. 2001).

Boswellia sacra Flueck. (Frankincense, Olibanum) (synonyms: *B. carterii*, *B. undulata*, *B. crenata*) belongs to the Burceraceae family, which includes several species growing in the Arabian Peninsula, India and East Africa (Camarda et al. 2007; Hasson et al. 2011). *B. sacra* from Dhofar Mountains are currently and ecologically relevant species showing symptoms of decline due to anthropic factors and possibly global warming (Liu et al. 2010; Raffaelli 2010). It provides several good services as timber, fodder, nectar and gum which are useful in traditional medicines, religious ritual and income regeneration (Eshete et al. 2012). Frankincense (gum, olibanum) is useful in pharmaceutical industry, flavoring, beverage, liqueurs, cosmetics, detergents, creams and as perfumery (Lemenih and Teketay 2003a,b; Eshete et al. 2012). Anticancer, anti-inflammatory, immunomodulatory, antimicrobial and anti-viral activities of several *Boswellia* species in addition to being a rich source of non-volatile triterpenoic constituents have been reported (Bűchele et al. 2003; Mothana and Lindequist 2005; Akihisa et al. 2006; Banno et al. 2006; Mothana et al. 2007, 2009; Hasson et al. 2011).

Epiphytic or endophytic fungi spend some part of their life cycle on or inside leaf tissues without negative impact (Farr et al. 1989; Elamo et al. 1999; Strobel 2002; Devarajan et al. 2002; Gamboa and Bayman 2006; Arnold 2007; Huang et al. 2008; Liu et al. 2010; Jalgaonwala et al. 2011). Numerous fungi have been isolated from different tissues of leaves and stems of terrestrial and aquatic plants as epiphytes or endophytes (Huang et al. 2008). Some of these endophytes might promote growth and ecological adaptability of the host by enhancing plant tolerance to environmental stress and resistance to phytopathogens and/or herbivores (Clay and Schardl 2002; Waller et al. 2005; Barrow et al. 2007; Liu et al. 2010; Sun et al. 2011). Therefore, the deterioration of beneficial endophytes could lead to the development of new disease problems (Mmbaga and Sauve 2009).

Numerous fungi have been isolated from unexplored sites, habitats, and substrates of extreme environmental conditions (Ilyas et al. 2009), however, there are many plant species from which endophytes have not yet been

isolated (Strobel 2002; Huang et al. 2008). The variations of endophytes/epiphytes diversity are due to generic variations among plants and the environmental conditions (Elamo et al. 1999). In Oman, little research has been carried out on coprophilous fungi (Gene et al. 1993; Elshafie 2005), aflatoxins and mycotoxigenic moulds (Elshafie and Al-Shally 1998; Elshafie et al. 1999; 2002), nematophagous fungi (Elshafie et al. 2003, 2005), endophytes (El-Nagerabi et al. 2013) and some plant diseases (Al-Bahry et al. 2005; Elshafie and Ba-Omer 2001). Nonetheless, there is no published study on the biodiversity of the fungal flora of the wild and cultivated plants of Dhofar Mountains, Oman. Endophytic fungi which are associated with wild and cultivated plant in Oman have not yet been extensively explored (El-Nagerabi et al. 2013).

Endophytes are among the poorly understood groups of fungi (Gazis and Chaverri 2010). It is quite promising to explore interesting and diverse fungal species among these plants. In the present investigation, we examined the diversity of the endophytic fungi colonizing *B. sacra*, collected from Dhofar Mountains during the growing season between May 2008 and October 2011; the fungal communities in the leaf and stem tissues were evaluated for their tissue-preference and seasonal variation.

MATERIALS AND METHODS

Sampling site

This study was carried out in Dhofar Mountains, Oman which is located at the South of the Arabian Gulf, bordered by Yemen on the South, the Arabian Sea on the Southeast, Iran on the Northeast, the United Arab Emirates on the Northwest, and Saudi Arabia on the West. It is located between latitude of 21°00'N-29°00'N and longitude of 51°00' E-59°40' E (Figure 1). The climate is hot-dry in the interior, hot-humid in the coastal area and humid in the south with summer monsoon rain. The average temperature is about 26°C with annual precipitation of less than 100 mm (AlKhanjari 2005). Dhofar mountain range is located in a coastal region known as "Nejd" covered by drought deciduous broadleaf anogeissus forest typical for the cloud oasis of the Dhofar Governorate in Oman (Miller and Morris 1988; Kurschner et al. 2004; Hildebrandt et al. 2006). The site is 500 m above the sea level and about 25 km away from the coast with 113-115 mm precipitation at the coast and a temperature of between 24-26°C, while they are 252 mm and 21°C near the mountain crest of 880 m elevation. The wet season, which is known as '*khareef*' in Arabic, is in summer from mid-June through mid-September when moist air from the Indian Ocean is pushed against the coastal mountains range, leading to orographic clouds and drizzle (Hildebrandt et al. 2006). During the rest of the year, desert climate prevails, beside, '*khareef*' rain, precipitation is rare and erratic, mainly from cyclones occurring about once in each three years (Brook and Cohen 2000).

Plant material

Duplicate samples of healthy green leaves and stems of *Boswellia sacra* were collected from Dhofar Mountains, Oman. The selected plant was identified at the Department of Biological Sciences and Chemistry, College of Arts and

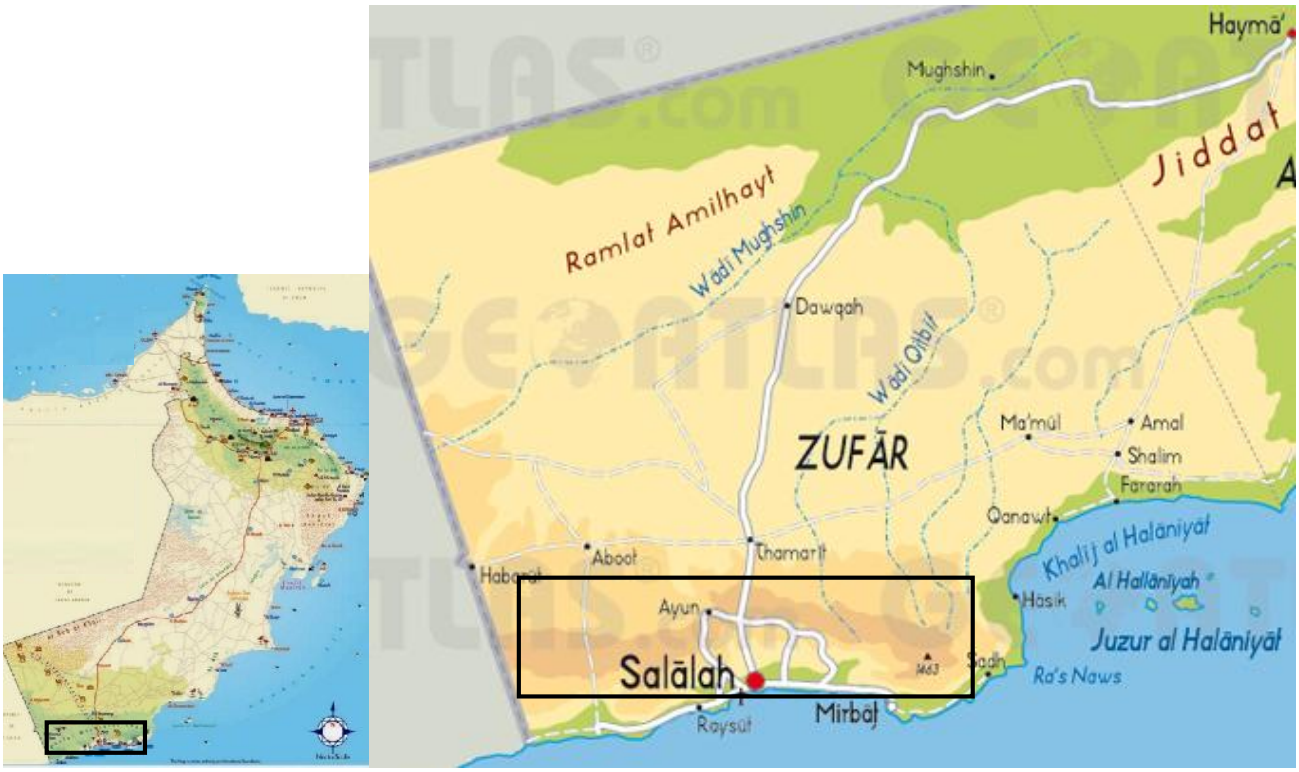

Figure 1. Sampling site in Dhofar (Zufar) Mountains, Oman

Sciences, University of Nizwa, and Department of Biology, College of Science, Sultan Qaboos University, Oman. Samples were collected at different times and seasons between 2008 and 2011. The samples were collected in sterile polyethylene bags and stored for less than a week in refrigerator at 5°C before testing.

Isolation of endophytic fungi

The tissues of the selected plant parts were cut into small pieces of approximately 1.0 × 0.5 cm in diameter and washed with several changes of sterile distilled water. The pieces were surface disinfected with 70% ethanol for 1 min followed by 5% sodium hypochlorite for 5 min (Gazis and Chaverri 2010; Liu et al. 2010). The disinfected pieces were aseptically inoculated on Potato Dextrose Agar (PDA, Potato, 200 g; dextrose, 20 g; agar 15 g; distilled water, 1L) supplemented with 0.05 mg/ml chloramphenicol to suppress the bacterial growth, and enable the mycelia development on the plant tissues. The inoculated plates were incubated at ambient temperature (27-29°C) for 7-10 days until the mycelium was apparent on the growth media. The developing fungal colonies were then inoculated on Petri dishes containing Malt Extract Agar (MEA) for preparation of pure colonies, identification and preservation as dry herbarium materials. Duplicates of the isolated fungi were deposited at the herbarium of Department of Biological Sciences and Chemistry, College of Arts and Sciences, University of Nizwa, and Department of Biology, College of Science, Sultan Qaboos University, Oman.

Identification of the isolated fungi

The fungal isolates were identified using macroscopic features based upon colony morphology on the growth media and microscopic observations of mycelia, asexual conidia and sexual spores according to different taxonomic books, monographs and taxonomic papers (Barnett 1955; Raper and Fennell 1965; Pitt 1979; Ellis 1971, 1976; Sutton 1980; Webster 1980; Nelson et al. 1983; Barnett and Hunter 1998, 2003).

Data analysis

The number of times each fungal species was isolated (NCI = Number of Cases Isolation) was calculated according to the formula used in our previous study (El-Nagerabi et al. 2013) as the number of the samples from which the fungus was isolated, whereas the occurrence remarks (OR) as a total number of the samples from which a given species was isolated compared to the total number used for the isolation of the fungi. The number of the samples from which a given species was isolated divided by the total number of the collected samples was used to calculate the incidence percentages of the fungal species.

RESULTS AND DISCUSSION

Biodiversity of endophytic fungal consortia/composition of the plant

Forty-three species and three varieties of fungi belonging to 15 genera and sterile mycelia were recovered from green leaves and stems of *Boswellia sacra* (Table 1). Different genera were identified with variable number of species such as *Alternaria* (12 species), which followed by *Fusarium* (9 species), *Aspergillus* (5 species and 3 varieties), *Cladosporium* and *Drechslera* (3 species), *Chaetomium* (2 species) and one species from each of the remaining 9 genera (*Botryodiplodia, Curvularia, Mucor, Penicillium, Phoma, Rhizopus, Scytalidium, Taeniolella, Ulocladium)* along with numerous unidentified species of the genus *Aspergillus, Cladosporium, Fusarium* and *Penicillium*. Of these fungi, *Alternaria* is the most prevalent (12 species, 12.5-83.3%) followed by *Aspergillus* (6.9-86.1%), *Mycelia sterilia* (76.4%), *Rhizopus stolonifer* (62.5%), *Drechslera* (40.3-54.2%), *Cladosporium* (20.8-52.8%), *Curvularia lunata* (38.8%), *Chaetomium* (15.3-26.3%), *Mucor hiemalis* (19.5%), *Penicillium* (9.8-27.8%), *Fusarium* (6.9-27.8%), *Ulocladium consortiale* (27.8%), whereas the remaining species (*Scytalidium thermophilum, Phoma solani, Taeniolella exilis* and *Botryodiplodia theobromae*) displayed low levels of occurrence (4.2-11.1%) (Table 1). Since this is the first study on the mycoflora of *B. sacra*, therefore all of the isolated fungi (43 species and 3 varieties) are new endophytes to the tissues of this plant, whereas 35 species are new records to the mycoflora of Oman and 12 species are new fungal flora to Arabian Peninsula (Table 1).

The plant tissues, specially leaves and stems are excellent reservoirs for several types of microorganisms including endophytic fungi (Petrini 1991; Bokhary et al. 2000). Endophytic fungi were continuously isolated from the tissues of the most parts of terrestrial and aquatic plants (Devarajan et al. 2002; Huang et al. 2008). They are important and quantifiable component of fungal community affecting plants biodiversity and structures (Krings et al. 2007; Huang et al. 2008). Several studies of endophytic fungi from tropical and temperate forests support the high estimate of species diversity (Kumar and Hyde 2004; Santamaria and Bayman 2005; Santamaria and Diez 2005; Sänchez-Märquez et al. 2007). Almost all the terrestrial plants studies have observed mitosporic, ascomycetes fungi and sterile forms as endophytes (Bills 1996; Devarajan et al. 2002). In present study, pigmented dematiaceous hyphomycetes and ascomycetes colonized the leaf and stem tissues of *B. sacra* (Table 1). Some of these ubiquitous fungi including the species of *Alternaria alternata, A. angustiovoide, A. brassicicola, Cladosporium, Helminthosporium, Chaetomium, Drechslera, Aspergillus, Fusarium, Penicillium, Phoma, Ulocladium,* and *Camarosporium* were isolated in similar study from many other plants (Huang et al. 2008; Sun et al. 2011). The dark mycelia of some of these fungi benefit their host through absorption of more UV radiation comparable to white mycelia (Sun et al. 2011). Therefore, these fungi might enhance the growth and improve ecological adaptation of the host plants by enhancing plant tolerance to environmental stresses and resistance to phytopathogens and/or herbivores (Clay and Schardl 2002; Waller et al. 2005; Barrow et al. 2007; Liu et al. 2010; Sun et al. 2011). It was concluded that the pigmented dark fungal mycelia increase the host resistance to microbes and hydrolytic

enzymes (Carlos et al. 2008; Sun et al. 2011).

Various fungal taxa were isolated as endophytes from the leaf tissues of single species of tropical plant (Petrini 1991). Some of these fungi are either pathogenic or saprophytic which obtained their nutrition from leaf exudates, insect secretion or from organic matters deposited on the leaf surface (Last and Deighton 1965; Bokhary et al. 2000). The variation of foliar endophytes/ epiphytes is due to genetic differences among trees and the variations in the environmental conditions (Elamo et al. 1999). Although large numbers of endophytes were obtained, few species dominate the community (Petrini et al. 1992). Some species of *Alternaria, Colletotrichum* and *Fusarium* have been reported as endophytes for many plants (Liu et al. 2010). *Phoma, Cladosporium*, and *Fusarium* are frequently reported to occur as endophytes in terrestrial plants of the tropics (Brown et al. 1998). In Japan, *Alternaria* spp., *Cladosporium* spp., *Stemphylium* spp., and *Pleospora* sp. were dominant endophytes of *Salicornia europaea* (Sun et al. 2011). *Alternaria alternata*, *Cladosporium cladosporioides* and *Penicillium chrysogenum* are the most common endophytes isolated from halophytes of the Red Sea Coast of Egypt (El-Morsy 2000). *Aspergillus niger* was the dominant endophytic fungus in mangrove and legumes (Dorothy and Kandikere 2009). Dematiaceous fungi universally inhabit different ecological zones and play important ecological role for the survival of the plants. Many species of the genus *Aspergillus* such as *A. fumigatus*, and *A. niger* in addition to species of *Penicillium* and *Fusarium* are adapted to different plant tissues (Ilyas et al. 2009). The colonization and isolation rates of the endophytic fungi isolated from 29 traditional Chinese medicinal and herbal plants ranged from36.7-100% and 0.45-1.75%, respectively (Huang et al. 2008). In the present investigations, some species of these endophytes such as *Alternaria alternata* (83.3%), *Aspergillus niger* (86.1%), *A. fumigatus* (25%), *Cladosporium cladosporioides* (20.8%), *Fusarium* spp. (6.9-27.8%), *Penicillium* spp. (9.8-27.8%), and *Phoma solani* (9.8%) were similarly recovered from the leaves and stems of *B. sacra* whereas the remaining species were reported for the first time as endophytes to this plant (Table 1).

Sterile mycelia consist of various morphological fungal types without any true spores. These fungi are considerably prevalent in endophytic investigations (Lacap et al. 2003). In similar studies of 29 medicinal plants, sterile mycelia had the highest relative frequency (27.2%) comparable to other endophytes (Huang et al. 2008). In the present study, 55 sterile mycelia were isolated from most of the tested samples and had the highest level of incidence (76.4%) and occurrence remark (Table 1). These mycelia revealed different macroscopic and microscopic features and do not form reproductive structures when incubated for long period of time to enhance sporulation as concluded in similar studies (Carlos et al. 2008; Sun et al. 2011).

Endophytic fungal community among different tissues

Many plants tissues are colonized by a characteristic population of microorganisms (Bowerman and Goos 1991). Endophytic fungi frequently demonstrate single host specificity at the plant species level, but this specificity could be influenced by seasonal changes of the climatic factors (Cohen 2004; Huang et al. 2008; Sun et al. 2011). Partial heterogeneity or geographic were used to indicate the endophytic fungal segregation impacted by environmental differences (Yahr et al. 2006). Recent study showed that endophytes are not host specific (Jalgaonwala et al. 2011) and could colonize multiple host species of the same plant family within the same habitat, and their distribution can be similar in closely related plant species (Huang et al. 2008). Single endophyte or strains of the same fungus can be isolated from different parts or tissues of the same host differ in their ability to utilize different substrates (Jalgaonwala et al. 2011). There are more species of endophytes from branches than leaves (Collado et al. 2000; Liu et al. 2010). In the study of *Suaeda corniculata*, 11 fungal species were isolated from the stem and 15 species were recovered from the leaves, whereas in *S. microphylla*, totally, 13 fungal species were isolated from the leaves and 18 species were recovered from the stems (Sun et al. 2011). These variations in endophytes colonization of branches and leaves could be caused by the difference of substrate and nutrients of the host tissues (Rodriguez 1994; Rodriguez et al. 2009; Sun et al. 2011). Endophytes which invade mature or senescent organs are less host-selective due to decreasing defense capabilities of aging plant tissues which associated with an increased nutrient supply for saprophytic taxa (Peršoh et al. 2010). Thus, the composition of fungal communities in aging leaves seems to be predominately resulted from contagious spread and depend on the spectrum of nearby sporulating fungal taxa. In the present study (Table 1), the leaves of *Boswellia sacra* were colonized by large number of endophytic species (43 species, 3 varieties) in comparison with stems (25 species). This may be attributed to differences in the structural and nutritional composition of the leaves and stems of this plant as concluded in similar studies (Rodriguez 1994; Rodriguez et al. 2009; Sun et al. 2011). The most frequent endophytic fungal taxa from 29 medicinal plants had a nearly ubiquitous presence in leaves and the stem of these plants (Huang et al. 2008). Some species of *Alternaria, Colletotrichum* and *Fusarium* have been reported as endophytes for many plants (Liu et al. 2010). *Phoma*, *Cladosporium*, and *Fusarium* are frequently reported to occur as endophytes in terrestrial plants of the tropics (Brown et al. 1998). The species of the genus *Alternaria, Cladosporium, Stemphylium*, and *Pleospora* were dominant endophytes of *Salicornia europaea* in Japan (Sun et al. 2011). *Alternaria alternata*, *Cladosporium cladosporioides* and *Penicillium chrysogenum* are the most common endophytes isolated from halophytes of the Red Sea Coast of Egypt (El-Morsy 2000). *Aspergillus niger* was the dominant endophytic fungus in mangrove and legumes (Dorothy and Kandikere 2009). It evident that dematiaceous fungi universally inhabit plants in different ecological zones and of important ecological role for the survival of the plants. Generally many species of the genus *Aspergillus* such as *A. fumigatus*, and *A. niger* in addition to species of *Penicillium* and *Fusarium* are adapted to different plant tissues (Ilyas et al. 2009). In the present

Table 1. Number of cases isolation (NCI, out of 36 samples) and occurrence remarks (OR) incidence percentages (I%) of endophytic fungi isolated from different tissues of *B. Sacra*

Isolates	Isolate type	NCI				
		Leaves	OR	Stems	OR	I%
Alternaria alternata	β	36	H	24	H	83.3
Alternaria brassicicola	β õω	30	H	14	M	61.1
Alternaria chartarum	β õ	16	M	-	-	22.2
Alternaria cheiranthi	β õω	11	M	-	-	15.3
Alternaria chlamydospora	β õω	27	H	7	L	47.2
Alternaria citri	β õω	23	H	14	M	51.4
Alternaria dianthi	β õω	32	H	-	-	44.4
Alternaria pluriseptata	β õω	31	H	9	M	55.6
Alternaria radicina	β õω	9	M	-	-	12.5
Alternaria raphani	βõ	33	H	12	M	62.5
Alternaria tenuis	βõ	27	H	-	-	37.5
Alternaria tenuissima	βõ	14	M	16	M	41.6
Aspergillus spp.		26	H	2	R	38.9
*Aspergillus flavus*var.*flavus*	β	21	H	4	R	34.7
Aspergillus flavus var. *columnaris*	β	14	M	-	-	19.4
Aspergillus fumigatus	β	15	M	3	R	25
Aspergillus nidulans	β	9	M	2	R	15.3
Aspergillus niger	β	36	H	26	H	86.1
Aspergillus terreus	β	11	M	-	-	15.3
Aspergillus terreus var. *terreus*	β	5	R	-	-	6.9
Botryodiplodia theobromae	βõ	3	R	-	-	4.1
Chaetomium globosum	βõ	11	M	-	-	15.3
Chaetomium spirale	βõ	15	M	4	R	26.3
*Cladosporium*spp.		18	M	3	R	29.1
Cladosporium cladosporioides	βõ	15	M	-	-	20.8
Cladosporium oxysporum	βõ	23	H	15	M	52.8
Cladosporium tenuissimum	βõ	21	H	-	-	29.1
Curvularia lunata	βõ	13	M	15	M	38.9
Drechslera australiensis	βõ	28	H	11	M	54.2
Drechslera hawaiiensis	βõ	28	H	5	R	45.9
Drechslera spicifera	β	20	H	9	M	40.3
Fusarium spp.		8	L	1	R	12.5
Fusarium chlaydosporum	βõ	20	H	-	-	27.8
Fusarium dimerum	βõ	7	L	-	-	9.8
Fusarium equiseti	βõ	15	M	2	R	23.7
Fusarium oxysporum	βõ	5	R	-	-	6.9
Fusarium pallidoroseum	βõ	8	L	-4	R	16.7
Fusarium pluriferatum	β õω	5	R	-	-	6.9
Fusarium poae	βõ	16	M	2	R	25
Fusarium semitectum	βõ	6	L	-	-	8.3
Fusarium solani	βõ	7	L	4	R	15.3
Mucor hiemalis	βõ	14	M	-	-	19.5
*Penicilliums*pp.		14	M	6	L	27.8
Penicillium auratiogriseum	β õω	7	L	-	-	9.8
Phoma solani	β õω	6	L	1	R	9.8
Rhizopus stolonifer	β	34	H	11	M	62.5
Scytalidium thermophilum	β õω	6	L	2	R	11.1
Taeniolella exilis	β õω	6	L	-	-	8.3
Ulocladium consortiale	βõ	17	M	3	R	27.8
Sterile mycelia		36	H	19	H	76.4

Note: β: New record for *B. sacra*, Õ: New record to the mycoflora of Oman, ω: New record to Arabian Peninsula, OR: Occurrence remarks, out of 36 samples, H: High, more than 18 samples, M: Moderate, between 9-18 samples, L: Low, between 6-8 samples, R: Rare, less than 6 samples.

study, some of these fungi were isolated from the leaves; other species were encountered on the stems, whereas the remaining fungi were prevailed on both tissues (Table 1). This variation in endophytes on different tissues types might suggest the tissue-preference of dominant individual fungus (Wilder and Müller 1984) or reflects their compatibility to colonize specific tissues (Rodriguez 1994; Rodriguez et al. 2009).

Seasonal diversity of endophytic fungi

Little is known about the temporal changes in the endophytic fungal community. Endophytic fungi recovered from the selected plant are similar during summer (March-July) and winter (September-January). Almost the same species of fungi were recovered from the tissues of the plant, and there were no evident variations of the fungal flora with the seasons (El-Nagerabi et al. 2013). These fungal species colonize the tissues of the plant consistently during the growing season. This is may be due to the continuous growth of the mycelia within the tissues and production of new spores to invade new tissues (Sun et al. 2011). However, the abundance of endophytes varied among sampling times and did not increase over time. On the other hand, precipitation may influence the incidence of endophytes (Sahashi et al. 2000; Göre and Bucak 2007). More fungal endophytes developed in plant tissues in spring comparable to autumn and the higher rainfall in spring may enhance evidence dispersal of the fungal spores (Göre and Bucak 2007). It has been concluded that smaller and the more scattered the plant fragments sampled the higher the probability of approaching real diversity values of endophytic fungal communities (Gamboa and Bayman 2006). Endophytes which colonize healthy plant tissues are either remain dormant or produce more extensive but symptomless infection (Devarajan et al. 2002). In the present study, there is no evidence of seasonal variation in the endophytic fungi associated with the leaves and stems tissues of *Boswellia sacra* as suggested by many authors (Sun et al. 2011).

CONCLUSION

This investigation evaluated the diversity of endophytic fungi inhabiting *B. sacra* plant. From this study, 43 species and 3 varieties and sterile mycelia of fungi were isolated from the leaves and the stems of this plant. Some of these fungi are new records to this plant, to the mycoflora of Oman and the Arabian Peninsula. There is evident tissue-preference of the endophytes isolated from leaves and stems as expressed by

the incidence and occurrence remark of these fungi which is not associated with any seasonal fluctuation. This study is based mainly on macroscopic and microscopic characterization of the isolated fungi which does not allow the identification of abundantly isolated sterile mycelia. In the future studies, we should adopt many molecular methods which help in identification of non-sporulating fungal isolates.

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