BIODIVERSITAS
Volume 14, Number 1, April 2013
Pages: 10-16

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d140102

Endophytic fungi associated with *Ziziphus* species and new records from mountainous area of Oman

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Manuscript received: 8 January 2013. Revision accepted: 30 March 2013.

ABSTRACT

El-Nagerabi SAF, Elshafie AE, AlKhanjari SS. 2013. Endophytic fungi associated with Ziziphus *species from mountainous area of Oman and new records. Biodiversitas 14: 10-16. Ziziphus* species of the family Rhamnaceae grow extensively in arid and semi-arid regions. It is possible that the endophytic fungi associated with this plant might enhance the host resistance to the environmental impacts. The endophytic fungal population inhabiting the healthy leaves of *Z. spina-christi* and *Z. hajanensis* plants were determined from April 2008 to October 2011. The endophytic fungal communities varied between the two species, and 45 fungal species, 18 sterile mycelia and 12 yeasts were isolated from *Z. spina-christi*, whereas 35 fungi, 11 sterile mycelia and 5 yeasts were recovered from *Z. hajanensis* indicating tissue and species-specificity and without any seasonal variation among the endophytes. These endophytes are new to *Ziziphus* plants and 45 species are new to the mycoflora of Oman, whereas 27 species are new to Arabian Peninsula. The genus *Alternaria* was the most prevalent (19-81%) followed by *Aspergillus* (19-78%), *Rhizopus stolonifer* (78%), *Mycelia sterilia* (69%), yeasts (47%), *Cladosporium* (11-56%), *Drechslera* (14-53%), *Curvularia* (8-50%), *Fusarium* (6-33%), *Ulocladium* (41-31%), *Penicillium* (3-22%)), *Alysidium resinae* (11%), *Trichocladium* (6-11%), *Anguillospora longissima*, *Bactrodesmium rahmii*, *Catenularia* (8%), *Helminthosporium sorghi* (7%), *Dendryphiella infuscans* (6%), *Hansfordia biophila* (3-6%), *Arthrinium*, *Dissophora*, and *Phoma sorghina* (3%). The recovery of many fungal isolates, morphologically various sterile mycelia and yeasts suggests the high biodiversity of the endophytes invading these plants with strong evidence for future isolation of numerous fungal species through adopting more advanced molecular and DNA identification methods.

Key words: Al-Jabal Al-Akhdar, biodiversity, endophytic fungi, Oman, species-specificity, tissue-specificity, *Ziziphus spina-christi*, *Z. hajanensis*.

INTRODUCTION

Mountains are an important ecosystem attracting different interests of the world. They cover 24% of the earth surface and support 12% of the world population as an excellent source for water. They are inhabited by diverse flora and fauna (Anon 2008). In Oman, Al-Jabal Al-Akhdar in the Western Hajer mountains (1500 m) is a globally distinguished ecosystem having various climatic conditions and diverse vegetation. It has experienced rapid development which is associated with noticeable climatic changes and vegetation deterioration. The problem is not limited to conspicuous flora and fauna, but extends to fungi and bacteria which depend on higher plants for their survival (Carlile et al. 2001).

Ziziphus also known as "Sedra" is an important genus of the family Rhamnaceae found growing extensively in arid and semi-arid regions and represented by 135-170 species (Bhansali 1975; Mathur and Vyas 1995; Maraghni et al. 2010). Of these, only *Z. spina-christi* (L.) Wild and *Z. hajanensis* are common species inhabiting Al-Jabal Al-Akhdar and are indigenous to Oman with a wide ecological and geographical distribution growing under variety of environmental conditions and depression in deep sandy soil (Maraghni et al. 2010). They are an excellent source of food, fodder and fuel (Mathur and Vyas 1995). Recently the anti-inflammatory analgesic and antispasmodic activities were reported in rodents (Borgi et al. 2008; Borgi and Chouchane 2009).

Numerous and diverse fungi were isolated from the tissues of most parts of terrestrial and aquatic plants specially the leaves as endophytes (Huang et al. 2008). Endophytes are fungi or other microorganisms which spend at least part of their life cycle inside leaf tissues without causing immediate overt negative effect (Far 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). However, certain endophytic fungi might promote growth and improve the 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 alteration of beneficial endophytes could lead to the development of new and devastating disease to the host plant (Mmbaga and Sauve 2009). These endophytes produce innumerable and valuable novel secondary metabolites (Strobe 2002). They are an excellent source of a therapeutically important class of metabolites (Pietra 1997).

Worldwide, many researchers are collecting and isolating fungi from unexplored sites, habitats and substrates, particularly in extreme environmental conditions (Ilyas et al. 2009). However, of all world plants, it seems that only a few species have had their complete complement of endophytes studies (Strobel 2002; Huang et al. 2008). The variations of endophytes are due in part to generic differences among plants and the variations in environmental conditions (Elamo et al. 1999). In Oman, the research carried out until now focused on coprophilous fungi (Gene et al. 1993; Elshafie 2005), mycotoxins and mycotoxigenic moulds (Elshafie and Al-Shally 1998; Elshafie et al. 1999, 2002), nematophagous fungi (Elshafie et al. 2003, 2005), and some plant diseases (Elshafie and Baomer 2001; Al-Bahry et al. 2005). There are few studies on some plant diseases of cultivated crops in different areas of Oman, nonetheless, there is no single study on the biodiversity of the fungal flora of the wild and cultivated plants of Al-Jabal Al-Akhdar . It is evident that endophytes are among a poorly understood group of fungi (Gazis and Chaverri 2010). It is quite promising to explore interesting endophytic fungal species among the myriad plants including the main two species of the genus *Ziziphus* namely *Z. spina-christi*, and *Z. hajanensis* which have never been explored so far. Therefore, in the present study, the diversity of the endophytic fungi associated with *Z. Spina-christi* and *Z. hajanensis* were investigated during the growing seasons, between April 2008 and October 2011, in the mountain of Al-Jabal Al-Akhdar, and the seasonal variation, biological diversity, and species-specificity of these endophytes in the leaves of the two selected plant species were evaluated.

MATERIALS AND METHODS

Sampling site

This study was carried out in Al-Jabal Al-Akhdar mountain, Oman (Figure 1), which is located at the South of the Arabian Gulf. It is 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 lays between latitude of 21°00′N - 29°00′N and longitude of 51°00′E - 59°40′E. The climate varies according to variation in geographical regions which 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). Al-Jabal Al-Akhdar in the Western Hajer mountain range above 1500 m with average temperature on the plateau of 18.5°C which is much lower than that in the surrounding region (29°C) and relative humidity of 46%.

Plant materials

Eighteen samples of healthy green leaves from two different stands of *Ziziphus spina-christi* and *Z. hajanensis*

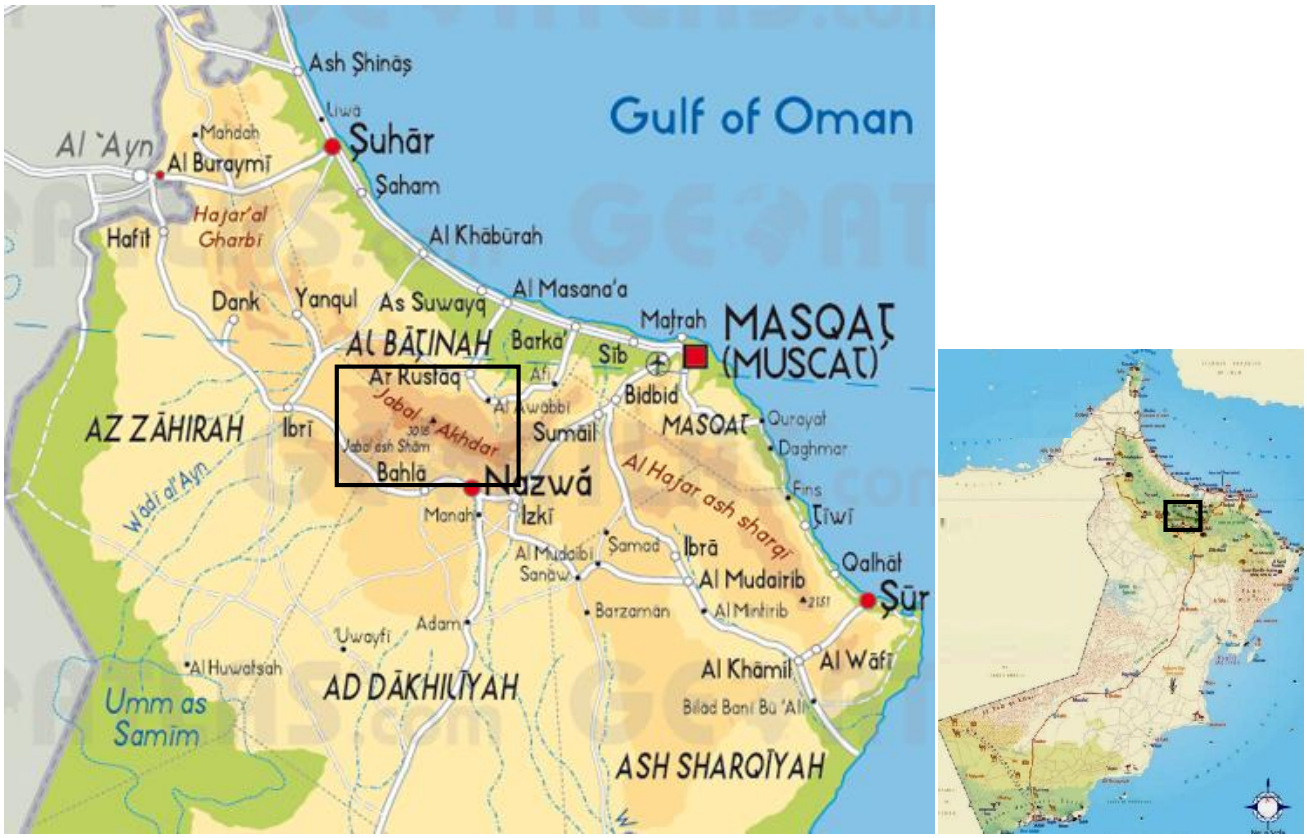

Figure 1. Sampling site in Al-Jabal Al-Akhdar mountain, Oman.

were collected at the same elevation of Al-Jabal Al-Akhdar mountain, Oman. The selected plants were identified at the Department of Biological Sciences and Chemistry, College of Arts and Sciences, University of Nizwa, and Department of Biology, College of Science, Sultan Qaboos University. Samples from 10 different plants were collected at different times and seasons between April 2008 to October 20011 (Table 1). The samples were kept in sterile polyethylene bags and stored in refrigerators at 5°C to be used for the isolation of the endophytic fungi.

Table 1. The collection date and plant material samples used for isolation of endophytic fungi from *Ziziphus spina-christi* and *Z. hajanensis*

Sample Nos.	Tissues used for isolation	Sample date
SM1	Green leaf	4-2008
SM2	Green leaf	6-2008
SM3	Green leaf	9-2008
SM4	Green leaf	12-2008
SM5	Green leaf	3-2009
SM6	Green leaf	5-2009
SM7	Green leaf	9-2009
SM8	Green leaf	11-2009
SM9	Green leaf	1-2010
SM10	Green leaf	3-2010
SM11	Green leaf	5-2010
SM12	Green leaf	7-2010
SM13	Green leaf	9-2010
SM14	Green leaf	12-2010
SM15	Green leaf	3-2011
SM16	Green leaf	5-2010
SM17	Green leaf	7-2010
SM18	Green leaf	10-2011

Isolation of endophytic fungi

The green leaves of the selected plant were cut into small pieces of 10 mm in length and washed with several changes of sterile distilled water. The pieces were surface disinfected with 70% ethanol for I min followed by 5% sodium hypochlorite for 5 min (Gazis and Chaverri 2010; Liu et al. 2010). The disinfected leaves were aseptically inoculated on Potato Dextrose Agar (PDA, Potato, 200g; dextrose, 20g; agar 15g; distilled water, 1L) supplemented with chloramphenicol (0.05 mg/ml) to inhibit the bacterial growth, until the mycelia appeared surrounding the plant tissues. The inoculated plates were incubated at the ambient temperature (27-29°C) for 7-10 days until the mycelial growth was apparent on the media. The fungal colonies which developed on the tissues were then inoculated on Malt Extract Agar (MEA) for preparation of pure colonies and further identification and preservation as dry herbarium materials at the herbaria 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.

Identification of endophytic fungi

The isolated endophytic fungi were identified using macroscopic features based upon colony morphology on the growth media and microscopic observations of mycelia and asexual/sexual spores according to the method described in literature, and consulting many taxonomic books and numerous monographs (Barnett 1955; Raper and Fennell 1965; Kobayashi 1970; Pitt 1979; Ellis 1971, 1976; Sutton 1980; Webster 1980; Nelson et al. 1983; Samson et al. 1995; Barnett and Hunter 1998, 2003; Barac et al. 2004).

Data analysis

The number of cases of isolation (NCI) of each fungal species was calculated according to modified formula of Gazis and Charerril (2010) 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 samples was used to calculate the percentage incidence of fungal species in each genus.

RESULTS AND DISCUSSION

Biodiversity of endophytic fungi

Fifty two species belonging to 21 genera of fungi in addition to unidentified 29 sterile mycelia and 17 yeasts were isolated from the green leaves of two *Ziziphus* species plants (*Z. spina-christi*, *Z. hajanensis*) (Table 2). Of these isolates, 45 species, 18 sterile mycelia and 12 yeasts were isolated form *Z. spina-christi*, whereas 35 species, 11 sterile mycelia and 5 yeasts were recovered from *Z. hajanensis*. The highest number of species were recovered from the genus *Alternaria* (9 species), followed by *Drechslera* (7 species), *Aspergillus* and *Fusarium* (6 species), *Cladosporium* (4 species), *Curvularia*, *Penicillium* (3 species), *Hansfordia*, *Trichocladium*, *Ulocladium* (2 species), and one species of *Anguillospora*, *Bactrodesmium*, *Catenularia*, *Dendryphiella*, *Helminthosporium* and *Rhizopus* along with an unidentified isolates from the genera of *Aspergillus*, *Dissophora*, *Fusarium*, and *Penicillium*. The species of the genus *Alternaria* was the most predominant genus on the leaf tissues and were isolated from 19-81% of the samples collected at different time of the year. This genus is followed by *Aspergillus* (19-78%), *Rhizopus stolonifer* (78%), sterile mycelia (69%), yeasts (47%), *Cladosporium* (11-56%), *Drechslera* (14-53%), *Curvularia* (8-50%), *Fusarium* (6-33%), *Ulocladium* (41-31%), *Penicillium* (3-22%)), *Alysidium resinae* (11%), *Trichocladium* (6-11%), *Anguillospora longissima*, *Bactrodesmium rahmii*, *Catenularia* (8%), *Helminthosporium sorghi* (7%), *Dendryphiella infuscans* (6%), *Hansfordia biophila* (3-6%), *Arthrinium*, *Dissophora*, and *Phoma sorghina* (3%). Since there is no previous study on the endophytic fungi of *Ziziphus* plants, the whole fungi isolated in the present investigation are new records to these plants, whereas 45 species were reported for the first time in the mycoflora of Oman and 27 species are new to the mycoflora of Arabian Peninsula (Table 2).

Table 2. Number of cases of isolation (NCI, out of 18 samples), occurrence remarks (OR) and incidence percentage (I%) of endophytic fungi of *Ziziphus spina-christi* and *Z. hajanensis*

Isolates	*Record type*	*Z. spina-christi*		*Z. hajanensis*		I%
		NCI	OR	NCI	OR	
Alternaria alternata	®*	18	H	7	M	69
Alternaria chlamydospora	®	13	H	6	M	53
Alternaria cheiranthi	® Ψ	7	M	-	-	19
Alternaria cineraniae	® Ψ	5	L	-	-	14
Alternaria citri	® Ψ	16	H	4	L	56
Alternaria pluriseptata	® Ψ	18	H	11	H	81
Alternaria radicina	® Ψ	14	H	9	M	64
Alternaria tenuissima	®	16	H	12	H	78
Alternaria triticina	® Ψ	11	H	-	-	31
Alysidium resinae	® Ψ	4	L	-	-	11
Anguillospora longissima	® Ψ	3	L	-	-	8
Arthrinium sp.	®	1	R	-	-	3
Aspergillus spp.	®	7	M	5	L	33
Aspergillus caespitosus	® Ψ	5	L	2	R	19
Aspergillus flavus	®*	14	H	8	M	61
Aspergillus fumigatus	®*	16	H	4	L	56
Aspergillus niger	®*	18	H	10	H	78
Aspergillus unguis	® Ψ	8	M	2	R	22
Aspergillus wentii	®	6	L	2	R	19
Bactrodesmium rahmii	®	-	-	3	L	8
Catenularia state of *Chaetosphaeria innumera*	® Ψ	2	R	1	R	8
Cladosporium spp.	®	8	M	3	L	31
Cladosporium chlorocephalum	® Ψ	-	-	5	L	14
Cladosporium cucumerinum	®	6	M	-	-	19
Cladosporium sphaerospermum	®	3	L	-	-	11
Cladosporium tenuissimum	®	12	H	8	M	56
Curvularia harveyi	® Ψ	3	L	-	-	8
Curvularia intermedia	® Ψ	5	L	2	R	19
Curvularia lunata	®*	10	H	8	M	50
Dendryphiella infuscans	® Ψ	2	R	-	-	6
Dissophora sp.	® Ψ	1	R	-	-	3
Drechslera australiensis	®	10	H	6	M	44
Drechslera biseptata	® Ψ	5	L	3	L	22
Drechslera hawaiiensis	®	9	M	7	M	44
Drechslera indica	® Ψ	6	M	4	L	28
Drechslera ravenelii	® Ψ	4	L	-	-	11
Drechslera spicifera	®*	11	H	8	M	53
Drechslera cactivora	® Ψ	-	-	5	L	14
Fusarium spp.	®	5	L	3	L	22
Fusarium chlamydosporum	®	7	M	5	L	33
Fusarium lateritium	®	3	L	-	-	8
Fusarium merismoides	® Ψ	2	R	-	-	66
Fusarium nivale	®	6	M	-	-	17
Fusarium reticulatum	®	3	L	-	-	8
Fusarium sambucinum	®	4	L	1	R	14
Hansfordia biophila	® Ψ	-	-	2	R	6
Hansfordia pulvinata	® Ψ	-	-	1	R	3
Helminthosporium sorghi	® Ψ	2	R	-	-	7
Penicillium spp.	®	6	M	2	R	22
Penicillium chrysogenum	®	2	R	-	-	6
Penicillium purpurogenum	®	3	L	1	R	11
Penicillium citrinum.	®	5	L	3	L	22
Phoma sorghina	®	1	R	-	-	3
Rhizopus stolonifer	®*	15	H	13	H	78
Trichocladium canadense	® Ψ	-	-	4	L	11
Trichodochium disseminatum	® Ψ	-	-	2	R	6
Ulocladium alternariae	® Ψ	7	M	4	L	31
Ulocladium consortiale	®	4	L	1	R	14
Yeasts	*	12	H	5	L	47
Sterile mycelia	®	18	H	11	H	69

Note: *: Known to mycoflora of Oman; Ψ: New record to Arabian Peninsula; ® New record to the *Ziziphus* spp.; OR: Occurrence remarks, out of 18 samples; H: High, more than 9 samples; M: Moderate, between 6-9 samples; L: Low, between 3-5 samples; R: Rare, less than 3 samples;

The surface of plant tissues, especially leaves, are excellent reservoirs of several types of microorganisms including numerous endophytic fungi (Petrini 1991; Bokhary et al. 2000). Therefore, many fungal species were continuously isolated from the tissues of the most parts of terrestrial and aquatic plants (Devarajan et al. 2002; Huang et al. 2008). These fungi represent an important and quantifiable component of fungal biodiversity and are known to affect the biodiversity and structures of plant communities (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 studied have mitosporic, ascomycetes fungi and sterile forms as endophytes (Bill 1996; Devarajan et al. 2002). The present study showed that pigmented dematiaceous hyphomycetes (mitosporic fungi) and ascomycetes colonized the tissues of these plant species (Table 2). Some of these fungi such as 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 of halophytic *Suaeda* spp. and medicinal plants from China (Huang et al. 2008; Sun et al. 2011). The dark mycelia of these fungi benefit their host through absorption of more UV radiation compared 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 as suggested by many authors (Clay and Schardl 2002; Waller et al. 2005; Barrow et al. 2007; Liu et al. 2010; Sun et al. 2011). Thus, it was suggested that the dark pigmented mycelia increase the host resistance to microbes and hydrolytic enzymes (Carlos et al. 2008; Sun et al. 2011).

Normally various fungal taxa were isolated as endophytes from the leaf tissues of single plant species of tropical plants (Petrini 1991). Some of these fungi are pathogenic or saprophytic which under favorable conditions may become pathogenic; while there are others which live on the leaves only as saprophytes and get their nutrition from exudates of the leaves' tissues, insect excretion or from air-borne organic matters deposited on the surface of the leaves (Last and Deighton 1965; Bokhary et al. 2000). The variations of foliar endophytes are due in part to genetic differences among trees and the variations in the environmental conditions (Elamo et al. 1999). In the investigation of species composition in woody plants, although large number of endophytes was obtained, few species dominated 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). *Alternaria* spp., *Cladosporium* spp., *Stemphylium* spp., and *Pleospora* sp. 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 is evident that dematiaceous fungi universally inhabit plants in different ecological zones and play important ecological roles 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 study, some of endophytic fungi isolated in similar studies (Petrini et al. 1992; Dorothy and Kandikere 2009; Ilyas et al. 2009; Sun et al. 2011) were recovered from the green leaves of the two species of *Ziziphus* plants whereas the remaining species were reported for the first time as endophytic fungi on these plant species (Table 2). These indicate the endophytic nature of fungi frequently isolated from the green leaves of these two *Ziziphus* species.

Biodiversity of sterile mycelia

Sterile mycelia consist of various morphological fungal types without any true spores. These fungi are considerably prevalent in endophytic investigations (Lacap et al. 2003; Huang et al. 2008). Of the frequently encountered endophytic fungal groups, sterile mycelia had the highest relative frequency (27.2%) (Huang et al. 2008). In the present study, 29 sterile mycelia were isolated from the tested samples with the highest occurrence remark and level of incidence (69%) (Table 2). These mycelia revealed different macroscopic and microscopic features and do not form reproductive structures when incubated for long period of time in order to enhance fungal sporulation. This suggests the high possibility of isolating more fungal species using advanced identification methods.

Endophytic fungal community among different plant species

Many plants 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; Hung et al. 2008; Sun et al. 2011). Partial heterogeneity or geographic separations were used to indicate the endophytic fungal segregation impacted by environmental differences (Yahr et al. 2006). A recent study showed that endophytes are not host specific (Jalgaonwala et al. 2011). They 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). A single endophyte or different strains of the same fungus can be isolated from different parts or tissues of the same host, which indicate their ability to utilize different substrates (Jalgaonwala et al. 2011). These variations in endophyte colonization could be caused by the difference in substrates and nutrients of the host tissues (Rodrigues 1994; Rodriguez et al. 2009). 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). This may be attributed to differences in the structural and nutritional composition of the plant tissues (Rodrigues 1994; Rodriguez et al. 2009; Sun et al. 2011). In the present study (Table 2), the green leaves of *Z. spina-christi* and *Z. hajanensis* were similarly colonized by 31 species of endophytic fungi of variable levels of occurrence, whereas 19 species were specific to *Z. spina-christi* and 7 species were isolated from *Z. hajanensis*. The incidence levels of these fungi are evidently higher in the green leaves of *Z. spina-christi* comparable to *Z. hajanensis*. These indicate the possibility of some degree of species-specificity to these fungi as suggested by many authors (Cohen 2004; Hung et al. 2008; Sun et al. 2011) and with similar recovery at different incidence levels of endophytic fungi in these closely related *Ziziphus* species (Huang et al. 2008; Jalgaonwala et al. 2011).

Seasonal biodiversity of endophytic fungi

Little is known about the temporal changes in the endophytic fungal community. The diversity of endophytic fungi recovered from the selected plant is similar during summer (March-July) and winter (September-January). Almost the same species of fungi were isolated from the tissues of the plant, and there are no evident variations of fungal flora with the seasons. These results showed that fungal species colonizing the tissues of the plant were consistent during the growing seasons. 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 than in autumn and the higher rainfall in spring may enhance evidence dispersal of the fungal spores (Göre and Bucak 2007). It has been suggested that the 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). Fungal endophytes that colonize healthy plant tissues either remain dormant or produce more extensive but symptomless infections (Devarajan et al. 2002). In the present study, there are no apparent seasonal variations among of endophytic fungi associated with the two selected species of the genus *Ziziphus* as concluded in similar studies (Sun et al. 2011).

CONCLUSION

We isolated 52 species of 21 genera of fungi, and 29 sterile mycelia and 17 yeasts from the green leaves of *Z. spina-christi*, *Z. hajanensis*. Some of these fungi are new records for the plants and/or to the mycoflora of Oman and Arabian Peninsula. There is no seasonal variation in the endophytic fungi; however, there is some degree of species-preference observed in the endophytic distribution as shown by the composition of the fungal community,

isolation frequencies and occurrence remarks. This study was conducted using classical taxonomic methods and identification techniques which do not facilitate the isolation of many fungi and identification of numerous yeasts and sterile mycelia. Therefore, our future studies should focus and utilize many molecular techniques which improve our research and knowledge of the biodiversity of the endophytic fungi.

ACKNOWLEDGEMENTS

We thank the Department of Biological Sciences and Chemistry, College of Arts and Sciences, University of Nizwa, and Department of Biology, College of Science, Sultan Qaboos University for providing space and facilities to carry this research. We thank the University of Nizwa Writing Center for proof reading the English of this manuscript.

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