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Fungi associated with ascocarps of desert truffles from different parts of Iran

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Abstract: Fungi associated with ascocarps of *Terfezia claveryi, Tirmania nivea, T. pinoyi, Picoa lefebvrei* and *P. juniperi* in Iran showed a wide range of genera and species in 94 samples collected during 2005–2009 from different regions including Fars, Systan and Baluchestan, Kerman, East Azarbaijan, Khuzestan, Kermanshah and Hormozgan provinces. Thirty two species belonging to 21 genera were recovered from ascocarps of truffles on two culture media. *Penicillium chrysogenum, P. citrinum, P. griseofulvum, P. brevicompactum, P. crustosum, P. oxalicum, Aspergillus carbonarius, A. niger, A. flavus* and *A. terreus* were the most common fungal species isolated on glucose–Czapek agar medium. The number of fungal species recovered on 20% NaCl–Czapek agar were less (4 genera and 10 species). The most abundant fungal genera belonged to *Penicillium* and *Aspergillus.* Also, *Paecilomyces lilacinus* and *Scopulariopsis halophilica* could grow on Czapek agar medium amended with 20 % NaCl. Other fungal species were not able to grow on this medium.

Keywords: *Terfezia claveryi, Tirmania nivea, T. pinoyi , Picoa lefebvrei, P. juniperi,* Iranian desert truffles

Introduction

Desert truffles are the hypogeous ascocarps of some ascomycetous mycorrhizae, which can be found in semi-arid lands of North Africa, South of Europe and the Middle East, including Iran (Abdalla *et al.*, 1979; Colinas *et al.*, 1994; Ferdman *et al.*, 2005; Mandeel *et al.*, 2007; Jamali and Banihashemi, 2010). Several species of *Terfezia* and *Tirmania* form mycorrhizal symbiosis mainly on roots of Cistaceae family, including different species of the genus *Helianthemum* (Diez *et al.*, 2002). These plants and their associated mycota may play a major role in the maintenance of Mediterranean shrub lands and xerophytic grasslands and preventing erosion and desertification (Honrubia *et al.* 1992). There is a great relationship among truffles as soil fungi with other soil microorganisms particularly soil bacteria and fungi (Mohawed *et al.*, 1997). So, other fungi might be truffle fungi competitors (Luppi, 1972). The truffle mycelia grow slowly on synthetic media, therefore, other fungi can easily delay or inhibit their mycelial growth by competition. Several investigators have isolated different fungi from dry tubers in many parts of the world (Luppi and Fontana, 1977; Mohawed *et al.*, 2001). In Egypt, Mohawed *et al.*, (2001) studied fungal contamination of dry tubers

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and isolated *Aspergillus* from 65% of the samples. In Italy, Luppi and Fontana (1977) found that *Penicillium* was the predominant genus isolated from *Tuber melanosporum*. Most of truffle ascocarps are susceptible to fungal growth as well as to mycotoxins. The numbers of microorganisms associated with ascocarps vary from a few hundreds to thousands per gram of ascocarp and are mostly located on the outer surface. In spite of several investigations on natural occurrence of fungal contamination in truffle ascocarps in many parts of the world (Ceruti and Luppi, 1971; Luppi and Fontana, 1977; Mohawed *et al.*, 2001), there is no comprehensive study in Iran. The main objective of this study was determination of desert truffles' mycoflora in different parts of Iran.

Materials and Methods

Collection of Samples

Ninety four well identified and fresh samples of different truffle species were collected from various parts of Iran including Fars, Lorestan, West and East Azarbaijan, Kermanshah, Kerman, Hormozgan, and Systan and Baluchestan provinces during early March to late April 2005-2009. Samples were harvested from rangelands dominated by *Helianthemum* and *Carex* species and were identified to species level based on their macro- and micromorphological features. Molecular studies were used to confirm morphological identification. Dry samples of all identified species were deposited in fungal collection of the Department of Plant Protection, College of Agriculture, Shiraz University, Iran. Totally 94 samples including one species of *Terfezia*, two of *Tirmania* and two of *Picoa* were collected (Table 1).

Sample Preparation

Fresh ascocarp slices (1gr) were washed in 100 ml sterile distilled water using a rocking shaker. One ml of the washate and grindings of ascocarps was placed in 9 cm sterile Petri plates. Glucose-Czapek agar and Czapek agar amended with 20% NaCl were used for isolation of glucophilic as well as halophilic fungi, respectively. These media were amended with rose bengal (45 µg/ml) and chloramphenicol (25 µg/ml). Inoculated plates were incubated at 25 ^{0}C for 7 days for glucophilic fungi and 10-15 days for halophilic fungi. Five plates were used for each sample. The growing fungi were counted and identified morphologically based on macro- and microscopic characteristics (Ainsworth and Bisby, 1961; Booth, 1971; Ellis, 1971; Raper and Fennell, 1977; Pitt, 1979; Moubasher, 1993; Samson *et al.*, 1995; Barnett and Hunter, 1998, Stolk and Samson, 2001).

Results and Discussion

Glucophilic Fungi

Thirty two species comprising 21 genera were isolated from ascocarps of *Terfezia claveryi*, *Tirmania pinoyi*, *T. nivea*, *Picoa lefebvrei* and *P. juniperi*, on glucose-Czapek agar at 25^{o}C among isolated genera, 7 were recoverd from *Terfezia clavaryi*, 8 from *T. pinoyi* and *T. nivea* and 6 genera from *P. lefebvrei* and *P. juniperi*. The gross total numbers of glucophilic fungi were 4979 colonies per 1g fresh tuber ascocarp (Table 2).

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Table 1 Location of natural truffle populations collected during 2005-2009 from different provinces of Iran.

Location	Collection date	Latitude	Longitude	Sample code	Phenetic species
East Azarbaijan	2009	37^0 26´.00 N	46^0 13´.00 E	A1, A2, A3, A4, A5, A6	*Terfezia claveryi, T. claveryi, T. claveryi, Tirmania pinoyi, T. pinoyi, T. pinoyi*
Cistan and Blauchestan (Khash)	2009	30^0 40´.412 N	60^0 51´.149 E	A7, A8, A9	*T. pinoyi, T. pinoyi, T. pinoyi*
Cistan and Blauchestan (Zabol)	2009	30^0 48´.439 N	60^0 54´.176 E	A10, A11, A12	*T. claveryi, T. claveryi, T. claveryi*
Fars (Fasa-Garbaigan)	2010	28^0 43´.00 N	53^0 58´.00 E	A13, A14, A15	*T. claveryi, T. claveryi, T. claveryi*
Fars (Darab)	2007	28^0 35´.665 N	54^0 41´.313 E	A16, A17, A18, A19, A20, A21, A22	*T. claveryi, T. claveryi, T. claveryi, T. claveryi, , Picoa juniperi, , P. juniperi, , P. juniperi*
Fars (Shiraz)	2009	29^0 51´.244 N	52^0 25´.318 E	A23, A24, A25, A26, A27	*T. claveryi, Picoa Lefebvrei, P. lefebvrei, P. juniperi, P. juniperi*
Fars (Larestan-Aghoseh)	2007	27^0 49´.346 N	53^0 25´.344 E	A28, A29, A30, A31, A32	*T. claveryi, T. claveryi, T. pinoyi, T. pinoyi, T. pinoyi*
Fars (Larestan-Arad)	2007	27^0 48´.915 N	53^0 28´.629 E	A33, A34, A35, A36	*T. claveryi, T. claveryi , T. pinoyi, T. pinoyi*
Fars (Fasa-Emamzadeh esmaeil)	2007	290 08´.246 N	530 24´.967 E	A37, A38, A39, A40, A41, A42	*T. claveryi, T. claveryi , P.lefebvrei, P. juniperi, P. juniperi, P. juniperi*
Fars (Larestan-Evaz 1)	2009	270 47´.905 N	530 49´.068 E	A43, A44, A45, A46	*T. claveryi, T. pinoyi, T. pinoyi, T. pinoyi*
Fars (Larestan-Kahnuyeh)	2009	270 55´.978 N	530 20´.791 E	A47, A48, A49, A50	*T. claveryi, T. claveryi, T. pinoyi, T. pinoyi*
Fars (Larestan-lozuyeh)	2009	270 55´.958 N	530 20´.783 E	A51, A52, A53, A54, A55	*T. claveryi, T. claveryi, T. pinoyi, T. pinoyi, T. pinoyi*
Fars (Shiraz-tol akhondi)	2006	290 50´.998 N	520 23´.250 E	A56, A57, A58, A59, A60, A61, A62, A63	*T. claveryi, T. claveryi, P. juniperi, P. juniperi, P. juniperi, P. juniperi, P.lefebvrei, P. lefebvrei*
Fars (Nurabad)	2009	300 11´.00 N	510 45´.00 E	A64, A65, A66, A67, A68	*T. claveryi, T. claveryi, P. juniperi, P. juniperi, P. juniperi*
Kerman (Cirjan-Haji abad)	2007, 2010	280 18´.376 N	550 53´.906 E	A69, A70, A71, A72, A73, A74, A75	*T. claveri, T. claveryi , T. claveryi , Tirmania nivea, T. nivea, T. nivea, T. nivea*
Kerman (Cirjan-Baghat)	2010	290 25´.124 N	550 40´.674 E	A76, A77, A78, A79, A80	*T. claveri, T. nivea, T. nivea, T. nivea, T. nivea*
Lorestan	2009	330 28´.60 N	490 10´.60 E	A81, A82, A83, A84	*T. claveryi, T. claveryi, P. juniperi, P. juniperi*
Hormozgan (Bastak)	2006, 2010	270 21´.00 N	540 27´.00 E	A85, A86, A87	*T. claveryi, T. claveryi, T. claveryi,*
Kermanshah	2009,2010	340 35´.00 N	470 48´.00 E	A88, A89, A90	*T. claveryi, T. claveryi, T. claveryi*
Kordestan	2009	350 21´.00 N	460 58´.00 E	A91, A92	*T. claveryi, T. claveryi,*
Kohkiluyeh Boirahmah	2009	310 221´.00 N	500 3´.00 E	A93, A94	*T. claveryi, T. claveryi,*

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Table 2 Average total counts (ATC), calculated per g tuber and number of cases of isolation (NCI), occurrence remarks (OR) and percentage of total fungi (PTF) of various fungal genera and species isolated from 94 samples on glucose-Czapek agar at 25°C.

No. of isolate	Genera and species	Glucose CZ-medium		
		ATC	NCI & OR	PTF
1	*Acremonium* sp.	43	16 L	0.86
2	*Alternaria* sp.	25	36 M	0.5
3.1	*Aspergillus carbonarius*	700	81H	14.05
3.2	*A. flavus*	150	42 M	3.01
3.3	*A. terreus*	100	39 M	2.00
3.4	*A. niger*	400	61H	8.03
3.5	*A.* sp	50	21 L	1.00
4	*Circinella circinans*	29	26 L	0.58
5	*Cladosporium herbarum*	63	61H	1.26
6	*Chaetomium* sp.	28	18 L	0.56
7	*Cunninghamella echinulata*	76	62H	1.52
8	*Clonostachys*	63	51 M	1.26
8.1	*C. rosea*	51	50 M	1.02
8.2	*C. solani*	12	14 L	0.24
9	*Dreschlera* sp.	29	21 L	0.58
10	*Eupenicillium crustaceum*	30	28 L	0.6
11.1	*Fusarium solani*	103	52 M	2.06
11.2	*F. oxysporum*	39	21 L	0.78
11.3	*Fusarium* sp.	2	23 L	0.04
12	*Mucor* sp.	33	30 M	0.66
13	*Geotrichum* sp.	27	22 L	0.54
14	*Paecilomyces lilacinus*	44	40 M	0.88
15.1	*Penicillium chrysogenum*	1070	92H	21.49
15.2	*P. citrinum*	420	72H	8.43
15.3	*P. crustosum*	230	44 M	4.61
15.4	*P. oxalicum*	226	36 M	4.53
15.5	*P. brevicompactum*	370	63 H	7.43
15.6	*P. griseofulvum*	384	40 M	7.71
16	*Pithomyces sp.*	15	20 L	0.3
17	*Rhizopus sp.*	41	28 L	0.82
18	*Scopulariopsis*	30	20 L	0.6
18.1	*S. brevicaulis*	30	20 L	0.6
19	*Syncephalastrum racemosum*	47	40 M	0.94
20	*Trichoderma harzianum*	48	40 M	0.96
21	*Ulocladium* sp.	23	20 L	0.46
22	Unknown	36	30M	0.72
	Gross total count	4979	-	-
	Number of genera	21	-	-
	Number of species	32	-	-

Occurrence remarks (OR): H: High occurrence between 61-94
M: Moderate occurrence between 30-60
L: Low occurrence between 1-29

Several investigators have isolated different fungi from dry tubers in many parts of the world (Luppi and Fontana, 1977; Mohawed *et al.*, 2001). In the present study, the most abundant fungal species isolated from fresh ascocarps of collected truffles were *Penicillium* species (54.2%). In Egypt, Mohawed *et al.*, (2001) showed that *Aspergillus* could be isolated from 65% of contaminated dry tubers. In Italy, Luppi and Fontana (1977) found that *Penicillium* was the predominant genus isolated from *Tuber melanosporum*. In the present study of the six *Penicillium* species isolated, *P. chrysogenum* and *P. citrinum* were the most prevalent species followed by *P. griseofulvum* and *P. brevicompactum*. The remaining species were less frequently isolated (Table 2). Most of *Penicillium* species recovered in current study were also reported from desert truffles in different parts of the world (Ceruti and Luppi, 1971; Luppi and Fontana, 1977; Mohawed *et al.*, 2001). *Penicillium crustosum, P. oxalicum* and *P. revicompactum* have been recovered from *Zea mays*, *Hordeum vulgare*, and *Arachis hypogaea* in Iran (Ershad, 2009). *Aspergillus* was the second most common fungus isolated from desert truffles in Iran. It was recovered from 65% of the samples and comprising 28% of total fungi isolated from ascocarps (Table 2). The most and the least abundant species of the aspergilla were *A. carbonarius*, *A. niger A. flavus* and *A. terreus*, respectively (Table 2).
Fusarium species were also isolated from 34 samples of the truffles (36%). Mohawed *et al.*, (2001) isolated *Fusarium* species in low frequency of one percent from desert truffles Other fungal genera including *Alternaria, Clonostachys, Fusarium, Mucor, Paecilomyces, Syncephalastrum, Cunninghamella* and *Trichoderma* were also isolated in this study (Table 2). *Acremonium* was recoverd only from *Picoa juniperi* and *Scopulariopsis brevicaulis* from *T. claveryi*. Also, *Circinella circinans, Clonostachys solani, Eupenicillium crustaceum, Syncephalastrum racemosum* and

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Scopulariopsis halophilica are new records for mycoflora of Iran.

Halophilic fungi

Ten species belonging to four genera were isolated from desert truffles on 20% glucose-Czapek agar amended with 20% salt at 25°C, of which *Aspergillus carbonarius*, *Penicillium chrysogenum* and *P. citrinum* were the most abundant fungi (Table 3).

Table 3 Average total counts (ATC), calculated per g tuber and number of cases of isolation (NCI), occurrence remarks (OR) and percentage of total fungi (PTF) of various fungal genera and species isolated from 94 samples on 20% NaCl glucose-Czapek agar at 25°C.

No. of isolate	Genera and species	20% NaCl CZ		
		ATC	NCI & OR	PTF
1.1	*Aspergillus carbonarius*	102	43 M	25.43
1.2	*A. flavus*	17	4 L	4.23
1.3	*A. terreus*	53	14 L	13.21
1.4	*A. niger*	8	6 L	1.99
2	*Paecilomyces lilacinus*	28	13 L	6.98
3.1	*Penicillium chrysogenum*	129	30 M	57.1
3.2	*P. citrinum*	113	18L	21.17
3.5	*P. brevicompactum*	83	15L	20.7
3.6	*P. griseofulvum*	45	11L	11.2
4	*Scopulariopsis halophilica*	23	7L	5.7
	Gross total count	601	-	-
	Number of genera	4	-	-
	Number of species	10	-	-

Occurrence remarks (OR): H: High occurrence between 61-94
M: Moderate occurrence between 30-60
L: Low occurrence between 1-29

Paecilomyces lilacinus and *Scopulariopsis halophilica* could be grown on Czapek agar medium amended with 20% NaCl. The other fungal species that were recovered on glucose-Czapek agar were not isolated on this medium. Mohawed *et al.*, (2001) isolated nine molds from desert truffles in Egypt. They found that the predominant fungi were *Aspergillus*, *Penicillium* and *Mucor*. *A. carbonarius* was recovered from 45.7% of the samples constituting 14.5% of total fungi. It was the most prevalent species isolated from desert truffles. In the present study, *Penicillium* species had the most abundances in truffle (0.037×10^4 colonies/g drytuber), followed by *Aspergillus*, *Paecilomyces lilacinus* and *Scopulariopsis halophilica* (0.018×10^4, 0.28×10^2 and 0.23×10^2 colonies/g dry tuber, respectively), on 20% NaCl Cz. medium. Generally, it was observed that some fungal genera or species could not be grown on NaCl Czapek agar.

The most discrepant truffle taxon was *T. claveryi* that showed a wide range of susceptibility to increased series of interrelated fungal species (1-5 fungi) due to genetic features of ascocarps. The present work indicated that examined specimens of desert truffles were contaminated with several glucophilic as well as halophilic fungi especially members of *Penicillium*, *Aspergillus*, *Fusarium*, *Cunninghamella* and *Scopulariopsis* genera. Most of these fungi are considered as competitors in culture media, can produce mycotoxins and some are plant pathogens.

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قارچ‌های همراه با آسکوکارپ دنبل‌های بیابانی از مناطق مختلف ایران

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چکیده: در بررسی قارچ‌های همراه با آسکوکارپ‌های پنج گونه دنبل بیابانی شامل *Terfezia claveryi, Tirmania nivea, T. pinoyi, Picoa juniperi* و *P. lefebvrei* طیف وسیعی از فلور قارچی، از ۹۴ نمونه آسکوکارپ که طی سال‌های ۱۳۸۴ تا ۱۳۸۸ از مناطق مختلف ایران شامل استان‌های فارس، سیستان و بلوچستان، کرمان، آذربایجان شرقی، خوزستان، کرمانشاه و هرمزگان جمع‌آوری شده بودند به‌دست آمد. ۳۲ گونه قارچی متعلق به ۲۱ جنس، از نمونه‌های آسکوکارپ، روی دو محیط کشت گلوکز – چاپک آگار و گلوکز – چاپک آگار حاوی ۲۰ درصد نمک طعام به‌دست آمد. *Penicillium chrysogenum*، *P. citrinum*، *P. griseofulvum*، *P. brevicompactum*، *P. crustosum*، *P. oxalicum*، *Aspergillus carbonarius*، *A. niger*، *A. flavus* و *A. terreus* معمول‌ترین قارچ‌های همراه با آسکوکارپ گونه‌های فوق بودند. تعداد گونه‌های قارچی جدا شده روی محیط گلوکز - چاپک آگار کم و شامل چهار جنس و ده گونه بود. *Penicillium* و *Aspergilus* فراوان‌ترین و دو گونه *Paecilomyces lilacinus* و *Scopulariopsis halophilica* به میزان بسیار کم روی این محیط کشت رشد کردند. دیگر گونه‌های قارچی قادر به رشد روی این محیط کشت نبودند.

واژگان کلیدی: دنبل‌های بیابانی ایران، *Terfezia claveryi*، *Tirmania nivea*، *T. pinoyi*، *Picoa juniperi*، *P. lefebvrei*

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