

Distribution of Zoosporic and Terrestrial Fungi in Accumulated Rainfall Water in Abha, South Western Region, Saudi Arabia

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Abstract: Twelve identified and three unidentified species representing five genera of zoosporic fungi were recovered from forty samples of accumulated rainfall water which were collected from Abha city, southwestern region in Saudi Arabia using sesame seeds baiting technique at $20\pm 2^{\circ}\text{C}$. *Allomyces anomulus*, *Aqualinderella fermentans* and *Pythium thalassium* were the most dominant species of zoosporic fungi. A total of forty three species in addition to one variety which belong to nineteen genera of terrestrial fungi were isolated from forty samples of accumulated rainfall water on both glucose- and cellulose-Czapek's Dox agar media at 28°C . Glucose Czapek's Dox agar medium was somewhat richest in the isolated species of terrestrial fungi compared with cellulose Czapek's Dox agar medium. Most of the recoverable genera and species of terrestrial fungi were similar on both of isolation media in spite of some species were isolated only once on one isolation medium. The most dominant terrestrial fungal taxa on both isolation media were: *Aspergillus niger*, *Rhizopus rhizopodiformis*, *A. flavus* and *Cladosporium cladosporoides*.

Key words: Zoosporic fungi, terrestrial fungi, rainfall water

Introduction

Several investigations have been carried out on the incidence and distribution of zoosporic fungi in freshwater habitats in different regions worldwide (Alabi, 1972; Rattan *et al.*, 1980; Misra, 1982; Klich and Tiffany, 1985; El-Hissy and Khallil, 1989; Paul *et al.*, 1994; Nejadattari, 2001). Little attention had been given for isolation of terrestrial fungi from water resources (El-Nagdy *et al.*, 1992; Bettucci and Roquebert, 1995; El-Nagdy and Nasser, 2000; Ali and Nasser, 2001). The freshwater fungi including those of strictly aquatic habitats and those of terrestrial habitats are commonly found in pools, ponds, lakes, rivers and streams (Willoughby, 1961; Apinis, 1964; Dick, 1966). The origin of terrestrial fungi in freshwater habitats may be through either living or dead animal or plant and soil or litter having been in contact with water (Park, 1972). They are also likely to originate from air or washed with rainwater (Sparrow, 1960). Research on the occurrence and distribution of zoosporic and terrestrial fungi in accumulated rainfall water in Saudi Arabia has been initiated especially in recent years (El-Nagdy *et al.*, 1992; El-Nagdy and Nasser, 2000; Ali and Nasser, 2001). Since the incidence and distribution of mycota (zoosporic and terrestrial fungi) in rainfall water accumulated in Abha city, southwestern region in Saudi

Arabia have not yet studied. So, the objective of this study is to explore the occurrence and distribution of zoosporic and terrestrial fungi from accumulated rainfall water in Abha city, southwestern region in Saudi Arabia.

Materials and Methods

Collection of water samples

Forty samples of accumulated rainfall water were collected randomly from different localities in Abha city, southwestern region, Saudi Arabia during Mars 2002. Water samples were collected in clean, sterile glass bottles (500 ml capacity each) and seven bottles were used for each water sample at different locations. Three of these bottles contained sterilized germinated (over night) sesame seeds which proved to be the best for baiting fungal zoospores or hyphal fractions. Glass bottles were then brought directly to the laboratory within few hours and were used for the recovery of zoosporic and terrestrial fungi. A glass bottle of each collected water sample was subjected to further chemical analysis. The temperature of the collected water samples were measured directly in situ during the time of sampling (16-22°C). The pH values of the collected rainfall water samples were measured in the laboratory using pH meter. Moreover, the total soluble salts and organic matter contents were determined according to Jackson (1958).

Recovery of zoosporic fungi

Water samples collected in sterile glass bottles contain sesame seeds were used for the recovery of zoosporic fungi. For baiting and recovery of zoosporic fungi from water samples, the samples containing sesame seeds were poured in sterilized 15 cm diameter Petri-dishes. These dishes (3 for each collected sample) were left at room temperature (20±2°C) for colonization of sesame seeds baits by zoospores of fungi as described by El-Hissy and Khallil (1989). Thereafter, the colonized seeds were transferred into other equivalent Petri-dishes (10 cm in diameter) containing sterilized distilled water to which crystalline penicillin (2000 units per liter water) was added (Roberts, 1963), for suppressing the growth of bacteria. The prepared dishes were then incubated at for fifteen days during which the dishes were daily examined. The number of cases of isolation and occurrence remarks of zoosporic fungal genera and species were determined on basis of 40 tested water samples. Total counts (density) of fungal populations were determined where the fungal species appearing on one plate was counted as one colony and rated to the number of seeds (30 seeds) for each water sample.

Isolation and recovery of terrestrial fungi

Water samples in sterile glass bottles which contain no sesame seeds were used for isolation and recovery of terrestrial fungi. For this purpose, one ml of each water sample was transferred (under aseptic condition) using sterile Menzies's (1957) dipper into each of six sterile Petri-dishes (10 cm diameter each). Modified Czapek's Dox agar medium in which glucose (10 g l⁻¹) or cellulose (20 g l⁻¹) was used for isolation of glucophilic and cellulose-decomposing fungi and three plates were used for each isolation medium. Martin's Rose Bengal was used as a bacteriostatic agent (Smith and Dawson, 1944). Plates were then incubated at 28°C for 15 days during which the

developing colonies were counted, examined and identified. Total counts were calculated per one ml water for each water sample.

Identification of zoosporic fungi

The following references were employed for the identification of genera and species of zoosporic fungi recovered during this investigation: Coker (1923), Johnson (1956, 1971), Waterhouse (1956), Sparrow (1960), Scott (1961), Karling (1977), Van der Plaats-Niterink (1981) and Fuller and Jaworski (1987).

Identification of terrestrial fungi

The identification of terrestrial fungal genera and species was based on the following key references: Raper and Thorn (1949), De Vries (1952), Oilman (1957), Raper and Fennell (1965), Fassatiava (1967), Simmons (1967), Ames (1969), Rifai (1969), Booth (1977), Ellis (1971) and Domsch and Gams (1972).

Results and Discussion

Regarding physicochemical analysis, it was found that the pH values of the accumulated rainfall water samples were in the acidic radical (4.5-5.0). The measured pH values of the collected water samples did not have any influence on the occurrence and distribution of the recoverable mycobiota during this investigation. In agreement with these results, El-Naady *et al.* (1992), El-Nagdy and Nasser (2000) and Ali and Nasser (2001) have obtained similar results during their studies on zoosporic and terrestrial fungi from accumulated rainfall water in other regions in Saudi Arabia. In addition, other workers (Paul *et al.*, 1984; El-Hissy and Khallil, 1989; El-Hissy and Oberwinkler, 1999) attained similar findings in different parts in the world. Also, the results revealed that the organic matter content of the collected rainfall water samples was low and fluctuated between 17.20 and 367.04 mg l⁻¹. and it had no effect on the incidence of both zoosporic and terrestrial fungi. Similarly, El-Hissy and Oberwinkler (1999) found that the organic matter content of water samples seem to have no effect on the incidence and distribution of the recovered zoosporic fungi from natural surface waters in Baden Wuerttemberg in Germany. In contrast to these results. some researchers (Khulbe, 1981; Misra, 1982; El-Hissy *et al.*, 1992 and Ali and Nasser, 2001) recorded a close direct interrelationship between the occurrence and distribution of zoosporic fungi and organic matter content of tested water samples. The total soluble salts of the collected rainfall water samples was generally low and ranged between 22.4 and 428.8 mg l⁻¹. Water samples characterized by relatively low total soluble salts were the richest in zoosporic and terrestrial fungal genera and species and vice versa. In accordance with these results, several reports (Padgett, 1976; Te Strake, 1980; Amon and Yei, 1982; El-Hissy and Khallil 1989; El-Hissy and Oberwinkler, 1999; Ali and Nasser, 2001) confirmed an inverse relationship between the distribution of zoosporic fungi and total soluble salts of water habitats. The results of physicochemical characteristics were also similar to some extent to those realized by Abdel-Hafez (1982 a, b); Abdel-Kader *et al.* (1983) and Moubasher and Mazen (1994) during their studies on terrestrial fungi recovered from water sources.

Table 1: Total counts (TC, calculated per 30 seeds for each sample), % total counts, number of cases of isolation (NCI, out of 40 rainfall water samples) and occurrence remarks (OR) of zoosporic fungal genera and species recovered from 40 accumulated rainfall water samples which were collected from during Mars 2002 using sesame seeds baiting technique at 20±2°C

Zoosporic fungal genera and species	Total counts (TC)	% total counts	Number of cases of isolation (NCI)	Occurrence remarks (OR)
<i>Achlya</i>	93	10.85	6	L
<i>A. proliferates</i> (Coker)	55	6.42	4	R
<i>Achlya species</i> (non-sexual)	38	4.43	2	R
<i>Allomyces</i>	213	24.85	20	M
<i>A. anomalus</i> Emerson	158	18.44	12	M
<i>A. macrogymis</i> Emerson & Wilson	36	4.20	6	L
<i>A. monspeliensis</i> Amaud	19	2.22	3	R
<i>Aphanomyces</i>	139	16.22	22	H
<i>A. laevis</i> de Bary	58	6.77	8	L
<i>A. scaber</i> d Bary	10	1.17	3	R
<i>Aphanomyces species</i> (non-sexual)	61	7.12	11	M
<i>Aqilalinderella</i>	86	10.04	12	M
<i>A. fermentans</i> Emerson & Weston	86	10.04	12	M
<i>Pythium</i>	326	38.04	31	H
<i>P. proliferum</i> de Bary	67	7.82	7	L
<i>P. rastratum</i> Butler	46	5.37	6	L
<i>P. thalassium</i> Atkins	93	10.85	12	M
<i>P. ultimum</i> Trow	33	3.85	3	R
<i>Pythium species</i> (non-sexual)	87	10.15	7	L
Total count	857			

Where: H=high occurrence (more than 20 samples out of 40) M=moderate occurrence (between 10-20 samples)
L=low occurrence (between 5-9 samples) R=rare occurrence (less than 5 samples)

Zoosporic fungi

Twelve identified in addition to three unidentified (non-sexual) species belonging to five genera of zoosporic fungi were recovered from forty samples of accumulated rainfall water (Table 1). *Pythium* and *Aphanomyces* were the most prevalent genera of zoosporic fungi while *Achlya* was the less frequent.

Pythium was the leading genus of zoosporic fungi and it was appeared in high frequency of occurrence (31 out of 40 water samples) matching 38.04% of total number of isolates. This genus contributed the broadest spectrum of species since it was represented by four identified in addition to unidentified species. *Pythium thalassium* was of moderate occurrence (12 water samples out of 40 counting 10.85% of total isolates) whereas unidentified species, *P. proliferum* and *P. rostratum* were of low occurrence (7, 7 and 6 samples, respectively representing 10.15, 7.82 and 5.37% of total counts of isolates, respectively) and *P. ultimum* was of rare occurrence (3 samples matching 3.85% of total isolates). Studies on zoosporic fungi in various water habitats in different parts of the world revealed that *Pythium* was almost the commonest genus. In this respect, Hoenk and Bock (1954) recovered *Pythium* in high occurrence from the surface water in Heath-moor Lake in Germany. Water samples collected from River Nile in Egypt (El-Hissy *et al.*, 1982). some ponds of Kharga oases (El-Nagdy and

Table 2: Total counts (TC, calculated per ml water), number of cases of isolation (NCI, out of forty rainfall water samples) and occurrence remarks (OR) of genera and species of terrestrial fungi on glucose- and cellulose-Czapek's which were isolated from forty rainfall water samples accumulated in Abha city, southwestern region, Saudi Arabia at 28°C

Genera and species of terrestrial fungi	Glucose		Sucrose	
	TC	NCI& OR	TC	NCI& OR
<i>Altemaria</i>	13	8L	8	5L
<i>A. alternata</i> (Fr.) Kreissler	10	6L	6	3R
<i>A. temdsima</i> (Kunze: Fr.) Wiltshir	3	2R	2	2R
<i>Aspergilliis</i>	173	37 H	104	31 H
<i>A. candidiis</i> Link: Fr.	8	3R	-	-
<i>A. clavatus</i> Desmazieres	8	3R	-	-
<i>A. flavus</i> Link: Fr.	43	15M	37	17 M
<i>A. flavus</i> var. <i>columnaris</i> Raper & Fennell	8	3R	5	2R
<i>A. japonicus</i> Saito	5	2R	9	4R
<i>A. niger</i> van. Tieghem	51	21 H	31	18 M
<i>A. ochraceus</i> Wilhelm	21	6L	8	2R
<i>A. oryzae</i> (Ahlburs) Cohn	11	5L	14	8L
<i>A. versicolor</i> (Wuillemin) Tiraboschi	9	3R	-	-
<i>A. wentii</i> Wehmer	9	5L	-	-
<i>Botryotrichum</i>	7	3R	-	-
<i>B. piluliferum</i> Saccardo & Marchal	7	3R	-	-
<i>Chaetomium</i>	26	12 M	28	15M
<i>C. globosum</i> Kunze	5	3R	11	8L
<i>C. olivaceum</i> Cooke & Ellis	21	9L	17	7L
<i>Cladosporium</i>	26	15M	8	3R
<i>C. cladosporoides</i> (Fres.) de Vries	26	15M	8	3R
<i>Drechslera</i>	11	8L	-	-
<i>D. specifera</i> (Bain.) von Arx	11	8L	-	-
<i>Emericella</i>	17	7L	11	5L
<i>E. nidulans</i> (Eidam) Vuill.	7	3R	8	4R
<i>E. quadrilineota</i> (Thorn & Raper) Benjamin	5	2R	3	1R
<i>E. rugulosa</i> (Thorn & Raper) Benjamin	3	1R	-	-
<i>E. violaceo</i> (Fennell & Raper) Malloch & Cain	2	1R	-	-
<i>Epicoccum</i>	5	2R	3	1R
<i>E. nigrum</i> Link	5	2R	3	1R
<i>Fusarium</i>	31	12 M	24	14 M
<i>F. equiseti</i> (Corda) Sacc.	8	3R	4	1R
<i>F. oxysporum</i> Schlechtendal	8	3R	9	5L
<i>F. solani</i> (Mart.) Sacchardo	15	6L	11	8L
<i>Humicola</i>	8	3R	5	2R
<i>H. griseo</i> Traaen	8	3R	5	2R
<i>Mucor</i>	24	14 M	7	4R
<i>M. circinelloides</i> Van Tiegh.	19	10 M	5	2R
<i>M. phimbens</i> Bonord	5	4R	2	2R
<i>Myrothecium</i>	-	-	8	2R
<i>M. roridum</i> Tode	-	-	8	2R
<i>Paecilomyces</i>	6	4R	-	-

Table 2: Continue

Genera and species of terrestrial fungi	Glucose		Sucrose	
	TC	NCI& OR	TC	NCI& OR
<i>P. variotii</i> Bainier	6	4R	-	-
<i>Pemcillium</i>	71	31 H	55	25 H
<i>P. brevicompactum</i> Dierckx	3	2R	2	1 R
<i>P. chrysogenum</i> Thorn	17	9L	9	5L
<i>P. duclaiixii</i> Delacroix	9	4R	-	-
<i>P. funiculosum</i> Thorn	14	6L	12	9L
<i>P. islandicum</i> Sopp	13	5L	14	6L
<i>P. waksmanii</i> Zaleski	15	8L	18	7L
<i>Phoma</i>	21	6L	5	3R
<i>P. herbarum</i> Westendrop	21	6L	5	3R
<i>Rhizopus</i>	23	18 M	4	2R
<i>R. rhizopodiformis</i> (Cohn) Zopf	23	18M	4	2R
<i>Stachybotrys</i>	20	11M	18	13 M
<i>S. chatarum</i> (Ehrenb.) Lindt.	20	11M	18	13 M
<i>Trichoderma</i>	14	10M	18	12 M
<i>T. hamatum</i> (Bon.) Bain.	10	7L	9	6L
<i>T. harzianum</i> Rifai	2	1R	6	4R
<i>T. viride</i> Pers. Fr.	2	2R	3	2R
<i>Ulocladium</i>	11	7L	-	-
<i>U. alternariae</i> Simm.	5	3R	-	-
<i>U. atrum</i> Preuss	6	4R	-	-
Total counts	502		306	

Where: H=high occurrence (more than 20 samples out of 40) M=moderate occurrence (between 10-20 samples)
L=low occurrence (between 5-9 samples) R=rare occurrence (less than 5 samples)

Abdel-Hafez, 1990) and from Rossetta and Damietta branches in Delta region. Lower Egypt (El-Hissy and Khallil, 1989) were found to be rich and predominated with *Pythium* species. In addition, Paul *et al.* (1994) isolated eight *Pythium* species of common occurrence in different water sources collected from western African nations; Togo and Benin. *Pythium* also appears cosmopolitan for stress water resources since it was also isolated from extreme water habitats as water of melting snow in Poland (Czeczuga, 1991-1992). It was also isolated (3 species) by El-Nagdy and Nasser (2000) in high incidence from accumulated rainfall water samples in the Riyadh region in Saudi Arabia.

Aphanomyces came behind *Pythium* and it was also recorded in high incidence (22 out of forty rainfall water samples) comprising 16.22% of total number of isolates. It was represented by unidentified species (moderate occurrence, 11 samples), *A. laevis* (low occurrence, 8 samples) and *A. scaber* (rare occurrence, 3 samples) counting 7.12, 6.77 and 1.17% of total isolates. Previous works by El-Hissy and Khallil (1989) and El-Hissy *et al.* (1992) in Egypt indicated *Aphanomyces* recovery from the surface water in the River Nile system but in moderate to low frequency of occurrence. *Allomyces* was isolated in moderate occurrence (20 out of 40 water samples) contributing 24.85% of total number of isolates. This genus included three species namely; *A. anomalus* (moderate occurrence, 12 samples), *A. macrogynus* (low occurrence, 6

samples) and *A. monspeliensis* (rare occurrence, 3 samples) counting 18.44, 4.20 and 2.22% of total counts of isolates, respectively. More or less similar results were also obtained by El-Hissy and Khallil (1989), El-Nagdy and Abdel-Hafez (1990) and El-Hissy *et al.* (1992) who indicated the occurrence of *Allomyces* species in various Egyptian water resources. *Allomyces* was encountered in a similar study as the most prevalent genus from accumulated rainfall water collected from Riyadh region (Saudi Arabia).

Aqualinderella was also of moderate incidence (12 out of 40 water samples) counting 10.04% of total isolates and it was represented by only one species namely; *A. fermentans*. *Aqualinderella fermentans* was previously recovered but in high incidence in the ponds and streams within the campus of Ibadan University, Nigeria (Alabi, 1972). It was also recorded by El-Hissy and Oberwinkler (1999) in low occurrence from the natural surface water in Baden Wuerttemberg, Germany and its density was higher in closed ponds compared with running water of rivers and streams. Moreover, El-Nagdy and Nasser (2000) isolated *Aqualinderella fermentans* in high frequency of occurrence from accumulated rainfall water in the Riyadh region (Saudi Arabia).

Achlya was recorded in low frequency of occurrence during this investigation and it was isolated from 6 out of 40 rainfall water samples contributing 10.85% of total number of isolates. It was comprised both *A. proliferoides* (4 water samples) and unidentified species (2 samples) which were of rare incidence representing 6.42 and 4.43% of total isolates. In this regard, *Achlya* was also recovered in variable frequencies and in greatest species diversity from different water types in sporadic countries in the world (Alabi, 1973 in Nigeria; Rattan *et al.* 1980 in Iraq, K-hch and Tiffany, 1985 in USA and Nejdattari, 2001 in Iran). However, *Achlya* had a moderate frequency and contributed the broadest spectrum of species (6 taxa) in the accumulated rainfall water in the Riyadh region, Saudi Arabia (El-Nagdy and Nasser, 2000).

Terrestrial fungi

A sum of forty three species in addition to one variety appertaining to nineteen genera of terrestrial fungi were isolated from forty samples of accumulated rainfall water on glucose- and cellulose-Czapek's Dox agar media at 28°C as presented in Table 2. Glucose Czapek's Dox agar medium was the richest in the isolated terrestrial fungi compared with cellulose Czapek's Dox agar medium (Table 2).

Glucophilic fungi (recovered on glucose-Czapek's Dox agar medium at 28 °C

Forty two species in addition to one variety representing nineteen genera of terrestrial fungi were isolated on glucose Czapek's Dox agar media at 28°C from forty samples of accumulated rainfall water. Both *Aspergillus* (9 species and one variety) and *Penicillium* (6 species) contributed the broadest spectra of the isolated species. They were the most dominant genera and were recorded in high occurrence (37 and 31 samples out of total 40 rainfall water samples, respectively) comprising 173 and 71 isolates, respectively out of 502 isolates. Seven genera of terrestrial fungi were isolated in moderate occurrence and these were *Fusarium*, *Trichoderma* (3 species each; 12 and 10 samples, respectively), *Chaetomiium*, *Mucor* (2 species each; 12 and

14 samples, respectively), *Cladosporium*, *Rhizopus* and *Stachybotrys* (one species each; 15, 18 and 11 samples, respectively out of forty samples) counting 31, 14, 26, 24, 26, 23 and 20 isolates of total 502 isolates. *Emmericella* (4 species, 7 samples), *Alternaria*, *Ulocladium* (2 species each; 8 and 7 samples, respectively), *Drechslera* and *Phoma* (one species each; 8 and 7 samples, respectively) were of low occurrence matching 17, 13, 11, 11 and 21 isolates, respectively out of 502 isolates. The remaining three genera were isolated in rare incidence and these namely; *Botryotrichum*, *Epicoccum* and *Paecilomyces* (one species each; 3, 2 and 4 samples, respectively out of 40 water samples) contributing 7, 5 and 6 isolates out of total 502 isolates.

Nearly, these genera and species of terrestrial fungi (Table 2) were also recovered, but in different frequencies of occurrence and counts, from accumulated rainfall water in different areas in Saudi Arabia (El-Nagdy *et al.*, 1992; El-Naady and Nasser, 2000; Alt and Nasser, 2001). Also, several authors (Hudson 1972; Sundaram 1977; Tan *et al.*, 1985; Bettucci *et al.*, 1993; Bettucci and Roquebert, 1995) recovered and isolated all fungal genera and species which were obtained during this study from water or soil in other regions worldwide.

Cellulose-decomposing fungi (recovered on cellulose-Czapek's Dox agar medium at 28 °C

Thirty one species in addition to one variety which belong to fifteen genera of terrestrial fungi were identified and isolated on cellulose-Czapek's agar medium at 28 °C from the tested forty rainfall water samples. The results presented in Table 2 show a similarity of the isolated species to those recovered on cellulose-Czapek's agar medium. *Aspergillus* (5 species and one variety) and *Penicillium* (5 species) were the most prevalent isolated genera of terrestrial fungi and they were of high incidence (31 and 25 water samples, respectively) counting 104 and 55 isolates, respectively out of 306 isolates. Four genera were recovered as of moderate frequency of occurrence and these were *Fusarium*, *Trichoderma* (3 species each; 14 and 12 samples, respectively), *Chaetomium* (2 species, 15 samples) and *Stachybotrys* (one species, 13 samples) representing 24, 18, 28 and 18 isolates, respectively. Only two genera were recovered and isolated in low incidence and these namely; *Emmericella* and *Alternaria* (2 species, 5 samples each) matching 8 and 11 isolates, respectively out of 306 isolates. The rest seven genera of terrestrial fungi were isolated in rare frequency of occurrence (1-4 water samples each). These genera were *Mucor* (2 species), *Cladosporium*, *Epicoccum*, *Humicola*, *Myrothecium*, *Phoma* and *Rhizopus* (one species each) contributing 8, 3, 5 and 4 isolates out of 306 isolates.

The recoverable species taxa of terrestrial fungi were also previously isolated with variable frequencies and in different counts from substrata including soil, mud, water and other sources in Saudi Arabia (El-Nagdy *et al.*, 1992; El-Nagdy and Nasser, 2000; Ali and Nasser, 2001) and other countries (Abdel-Kader *et al.*, 1983; Moubasher and Mazen, 1994). The great majority of the isolated genera and species of terrestrial fungi were proved to be cellulose-decomposing fungi (Malik and Eggins, 1970; Walsh and Stewart, 1971; Stewart and Walsh, 1972; El-Nagdy and Nasser, 2000; Ali and Nasser, 2001).

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