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Occurrence of Terrestrial Fungi in Accumulated Rainfall Water in Saudi Arabia

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Abstract. The incidence and distribution of terrestrial mycobiota in 60 samples of accumulated rainfall, collected from different sites in eastern and western regions in Saudi Arabia, was investigated. Forty-five species related to 21 genera of terrestrial fungi were recovered from these samples on glucose- (GCDA) and cellulose-Czapek´s Dox agar (CCDA) media at 28ºC. CCDA medium (38 species belonging to 20 genera) gave higher species diversity of terrestrial fungi as compared with GCDA (35 species belonging to 16 genera). The majority of the identified species were similar on both media, although some species were recovered only once on one of both media. *Aspergillus* and *Penicillium* were the most prevalent taxa of isolated terrestrial mycobiota on the two used media. Also, *Aspergillus* and *Penicillium* contributed the broadest spectra of the isolated terrestrial fungal species where they were represented by only 10 and 7 species, respectively, whereas several genera had only one species.

***Keywords*:** Terrestrial fungi, Rainfall water, Glucophilic, Cellulose-decomposing.

Introduction

The incidence of glucophilic and cellulose-decomposing fungi either in different soil habitats in Saudi Arabia or in different geographical parts of the world was intensively investigated [1-7]. Terrestrial fungi origin in freshwater habitats may be either through living or dead plant/animal material, soil or litter, having been in contact with water [8]. Also, they are likely to originate from air or washed with rainwater [9]. Several workers have reported the occurrence of terrestrial fungi in freshwater resources [8, 10-14]. The freshwater fungi including those of strictly aquatic habitats and those of terrestrial habitats are commonly found in pools, ponds, lakes, rivers and streams [15-17]. Although some research work had been conducted on the occurrence and distribution of terrestrial fungi in accumulated rainfall in Saudi Arabia [18-20], more work still be needed in order to investigate the incidence and distribution of terrestrial fungi in accumulated rainfall in other regions of Saudi Arabia that have not been studied. Thus, this work was carried out to study the occurrence and genus and species diversity of terrestrial fungi in accumulated rainfall of eastern and western regions of Saudi Arabia.

Materials and Methods

Collection of water samples

Thirty samples of accumulated rainfall (surface water) were collected directly after rainfall at random locations from each of eastern and western regions in Saudi Arabia during February 2003. These samples were collected in clean, dried, sterile, screwed-glass bottles (500 ml capacity each). Three bottles were used for each water sample and tested site. Two of these bottles were used for the recovery of inhabiting terrestrial fungi (one for each of glucose or cellulose CDA medium). The third bottle was used for the chemical analysis of the rainfall water characteristics. Water temperature was measured in situ (area of study) at the time of sampling and it ranged between 15-20ºC. The pH values of rainfall water samples were measured in the laboratory using pH meter. The total soluble salts and organic matter contents of the collected water samples were estimated as described by Jackson [21].

Recovery and isolation of terrestrial fungi

For the recovery and isolation of terrestrial fungi from the collected rainfall water samples, one ml of each water sample was poured under aseptic condition using sterile Menzies´s [22] dipper into each of six sterile Petri plates (10 cm diameter each). Modified Czapek´s Dox agar medium in which glucose (10 g/ l) or cellulose (20 g/ l) 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 [23]. Plates were then incubated at 28ºC 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.

Terrestrial fungi identification

The recovered genera and species of terrestrial fungi were identified according to the following references and monographs adopted by: [24-36]. The identification of genera and species of terrestrial fungi based mainly on their growth behaviors and morphological characters.

Results and Discussion

Physicochemical characteristics of rainfall water samples

The physicochemical analysis of accumulated rainfall water samples revealed that the pH values of these samples were in the acidic range (4.0-6.5). The pH values did not show any effect on the incidence and distribution of taxa of terrestrial fungi in these samples. Similar data were reported by El-Nagdy *et al.* [18], El-Nagdy and Nasser [19] and Ali and Nasser [20] in different sites in Saudi Arabia.

Results of organic matter content of the tested rainfall water samples showed that it was of low values and ranged between 17.19 and 288.05 mg/ l. The organic matter content had no influence on the occurrence and distribution of the isolated terrestrial fungal species. These data were almost in accordance with those reported by Moharram

et al. [13], El-Nagdy and Nasser [19] and Ali and Nasser [20].

Total soluble salts of the collected rainfall water samples were generally low and ranged between 31.60 and 313.8 mg/ l. The richest rainfall water samples with terrestrial fungal species had relatively low total soluble salts content and vice versa. Similar findings were also attained by Abdel-Hafez [2, 3], Abdel-Kader *et al.* [4], Moubasher and Mazen [7] and Ali and Nasser [20].

Occurrence of terrestrial mycobiota

Data presented in Table 1 show that 45 species representing 21 genera of terrestrial mycobiota were recovered from 60 samples of accumulated rainfall water on G- and C-CDA media at 28ºC. Also, using CCDA growth medium showed slightly more species diversity as compared to GCDA medium

Some species of these terrestrial fungi such as; *Aspergillus tamarii*, *A. clavatus*, *A. carbonarius*, *Emericella rugulosa*, *Penicillium janthinellum* and *P. frequentans* were detected only on GCDA medium, whereas other species; *Aspergillus wentii*, *Chaetomium globosum*, *C. spiralis*, *Cladosporium herbarium*, *Epicoccum nigrum*, *Fusarium moniliforme*, *Gliocladium roseum*, *Neurospora crassa*, *Paecilomyces terricola* and *Phoma glomerata* were recovered only on CCDA medium.

Table 1. Total counts (calculated per ml water, TC), number of cases of isolation, (NCI, out of 60 samples) and occurrence remarks (OR) of genera and species of terrestrial fungi on glucose- and cellulose-Czapek′s which were recovered from 60 accumulated rainfall samples in Saudi Arabia at 28ºC

Genera and species of terrestrial fungi	Glucose		Cellulose	
	TC	NCI & OR	TC	NCI & OR
Alternaria	9	5 R	12	8 L
A. alternata (Fr.) Kreissler	9	5 R	12	8 L
Aspergillus	166	40 H	91	32 H
A. candidus Link: Fr.	14	7 L	7	2 R
A. carbonarius (Bainier) Thom	5	4 R	-	-
A. clavatus Desmazieres	5	2 R	-	-
A. flavipes (Bain & Sart) Thom & Church	4	3 R	5	2 R
A. flavus Link: Fr.	31	15 M	16	10 L
A. fumigatus Fresenius	15	9 L	7	5 R
A. niger van. Tieghem	63	29 H	46	21 M
A. ochraceous Wilhelm	19	10 L	6	2 R
A. tamarii Kita	10	7 L	-	-
A. wentii Wehmer	-	-	4	3 R

Table 1. *Continued*

Genera and species of terrestrial fungi	Glucose		Cellulose	
	TC	NCI & OR	TC	NCI & OR
Botryotrichum	2	1 R	11	7 L
B. atrogriseum Van Beyma	2	1 R	11	7 L
Chaetomium	-	-	25	15 M
C. globosum Kunze	-	-	18	12 L
C. spiralis Zoph	-	-	7	3 R
Cladosporium	13	7 L	24	15 M
C. cladosporoides (Fres.) de Vries	13	7 L	19	12 L
C. herbarium (Pers.) Link ex Gray	-	-	5	3 R
Drechslera	1	1 R	8	7 L
D. halodes (Drechshera) Subram & Jain	1	1 R	8	7 L
Emericella	16	7 L	7	3 R
E. nidulans (Eidam) Vuill.	11	4 R	5	2 R
E. quadrilineata (Thom & Raper) Benjamin	3	2 R	2	1 R
E. rugulosa (Thom & Raper) Benjamin	2	1 R	-	-
Epicoccum	-	-	5	3 R
E. nigrum Link	-	-	5	3 R
Fennellia	11	7 L	5	2 R
F. flavipes Wiley & Simmons	11	7 L	5	2 R
Fusarium	19	12 L	31	22 M
F. equiseti (Corda) Sacc.	6	3 R	12	5 R
F. oxysporum Schlechtendal	13	9 L	18	16 M
F. moniliforme Sheldon	-	-	1	1 R
Gliocladium	-	-	3	2 R
G. roseum Bainier	-	-	3	2 R
Mucor	20	11 L	7	4 R
M. circinelloides Van Tiegh.	20	11 L	7	4 R
Myrothecium	5	2 R	11	8 L
M. verrucaria (Alb. & Sch.) Dit. Ex St.	5	2 R	11	8 L
Neurospora	-	-	7	5 R
N. crassa Shear & Dodge	-	-	7	5 R
Paecilomyces	6	2 R	20	9 L
P. terricola Onions & Barron	-	-	7	3 R
P. variotii Bainier	6	2 R	13	6 R
Penicillium	68	33 H	51	31 H

Table 1. *Continued*

Genera and species of terrestrial fungi	Glucose		Cellulose	
	TC	NCI & OR	TC	NCI & OR
P. chrysogenum Thom	11	8 L	14	9 L
P. citrinum Thom	18	15 M	19	17 M
P. frequentans Westling	4	3 R	-	-
P. glabrum (Wehmer) Westling	12	8 L	8	5 R
P. janthinellum Biourge	4	3 R	-	-
P. purpurogenum Stoll	8	6 R	5	5 R
P. waksmanii Zaleski	11	6 R	5	4 R
Phoma	-	-	11	9 L
P. glomerata (Corda) Wollenw. & Hochapfel	-	-	11	9 L
Rhizopus	28	23 M	12	9 L
R. rhizopodiformis (Cohn) Zopf	28	23 M	12	9 L
Trichothecium	4	2 R	6	4 R
T. roseum (Pers.) Link ex Gray	4	2 R	6	4 R
Trichoderma	23	18 M	32	23 M
T. hamatum (Bon.) Bain.	14	8 L	6	4 R
T. harzianum Rifai	5	4 R	5	3 R
T. viride Pers. Fr.	4	4 R	21	16 M
Ulocladium	9	5 R	-	-
U. atrum Preuss	9	5 R	-	-
Total counts	400		379	

H = High occurrence (more than 30 samples out of 60).
M = Moderate occurrence (between 15-30 samples).
L = Lower occurrence (between 7-14 samples).
R = Rare occurrence (less than 7 samples).

Glucophilic fungi

Thirty-five species belonging to 16 genera of terrestrial fungi were identified on GCDA medium (Table 1). The genera *Aspergillus* (9 species, 166 colonies out of 400 fungal total count) and *Penicillium* (7 species, 68 colonies) had the greatest diversity of the isolated species as well as the highest fungal total count (Fig. 1). They were the most prevalent genera (Fig. 1), hence they were rated of high occurrence (40 and 33 samples, respectively out of 60 tested rainfall water samples). Of the isolated species of *Aspergillus*; *A. niger* was of high (29 samples, 63 colonies), *A. flavus* was of moderate (15 samples, 31 colonies), *A. ochraceous*, *A. fumigatus*, *A. tamarii*, *A. candidus* (10, 9, 7, 7 samples, 19, 15, 10, 14 colonies, respectively) were of low and *A. carbonarius*, *A. flavipes* and *A. clavatus* (4, 3, 2 samples, 5, 4, 5 colonies, respectively) were of rare occurrence. *Penicillium* species were either of moderate (*P. citrinum*; 15 samples, 18 colonies), low (*P. chrysogenum* and *P. glabrum*; 8 samples each, 11 and 12 colonies, respectively) and rare (*P. frequentans*, *P. janthinellum*, *P. purpurogenum* and *P. waksmanii*; 3 – 6 samples and 4 – 11 colonies each) incidence.

Two genera namely; *Rhizopus* (*R. rhizopodiformis*, 23 samples, 28 out of 400 colonies) and *Trichoderma* (3 species, 18 samples, 23 colonies) were ranked as moderate frequency of incidence. Both *Fusarium* (*F. oxysporum* and *F. equiseti*) and *Mucor* (*M. circinelloides*) were ranked of low frequency of occurrence where they were isolated from 12 and 11 rainfall water samples, respectively (Fig. 1) contributing 19 and 20 colonies, respectively. *Emericella* and *Fennellia* were also of low incidence and they were recovered from 7 rainfall water samples (Fig. 1) each contributing 16 and 11 out of 400 colonies, respectively. *Emericella* was represented by three species, which rarely occurred (*E. nidulans*, *E. quadrilineata* and *E. rugulosa*) and was represented by only one species *Fennellia* (*F. flavipes*). The remaining genera of glucophilic fungi were isolated in rare incidence (1-5 out of 50 rainfall water samples each) and each genus was represented by only one species. These genera were; *Alternaria* (*A. alternata*), *Ulocladium* (*U. atrum*), *Myrothecium* (*M. verrucaria*), *Paecilomyces* (*P. variotii*), *Botryotrichum* (*B. atrogriseum*), *Trichothecium* (*T. roseum*) and *Drechslera* (*D. halodes*) matching 9, 9, 5, 6, 4, 2 and one colonies, respectively out of 400 total colonies.

General outlook on the recoverable genera and species of terrestrial mycobiota during this investigation reveal that almost these fungal taxa were also isolated with variable counts and frequencies from freshwater of the River Nile and Nile system in Egypt [37-39], from the Inler River in Ireland [10], on leaves submerged in a stream water in Canada [40], in freshwater ponds in India [11] and in wastewaters and stabilization pond [41]. The isolated species of terrestrial fungi were also found to be the main components of terrestrial mycobiota, with some minor differences, in accumulated rainfall water in other screened areas in Saudi Arabia [18-20].

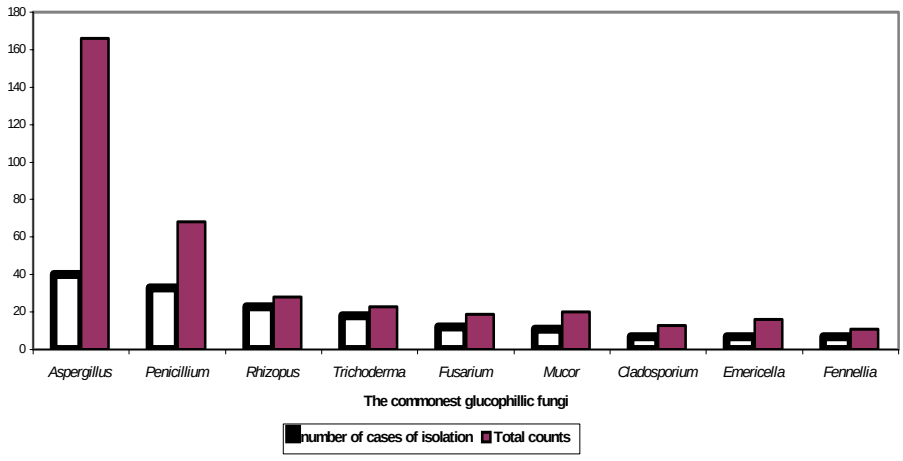

Fig. 1. Total counts (out of 400 isolates) and number of cases of isolation (out of 60 rainfall samples) of the most common terrestrial fungal genera recovered on GCDS medium at 20^{O}C.

Cellulose-decomposing fungi

As indicated in Table 1, 38 species appertaining to 20 genera of terrestrial mycobiota were identified on CCDA medium at 28ºC. It is interesting to find that *Aspergillus* (7 species, 32 samples, 91 colonies) and *Penicillium* (5 species, 31 samples, 51 colonies) were also the commonest genera of terrestrial mycobiota (highly occurred in rainfall water samples) isolated on CCDA medium (Fig. 2) and they were also contributed the broadest spectra of species as compared with the other genera. *Aspergillus* included seven species of which *A. niger* (21 samples, 46 colonies) was of moderate, *A. flavus* (10 samples, 16 colonies) was of low, *A. fumigatus*, *A. wentii*, *A. candidus*, *A. ochraceous* and *A. flavipes* were of rare occurrence (5, 3, 2, 2, 2 samples, respectively, 7, 4, 7, 6, 5 colonies, respectively) were of rare occurrence. Five species of *Penicillium* were identified and was found to be: *P. citrinum* of moderate incidence (17 samples), *P. chrysogenum*, of low (9 samples), *P. glabrum*, *P. purpurogenum* and *P. waksmanii* of rare incidence (each, 5, 5, 4 samples, respectively) representing 19, 14, 8, 5 and 5 out of 379 total fungal colonies.

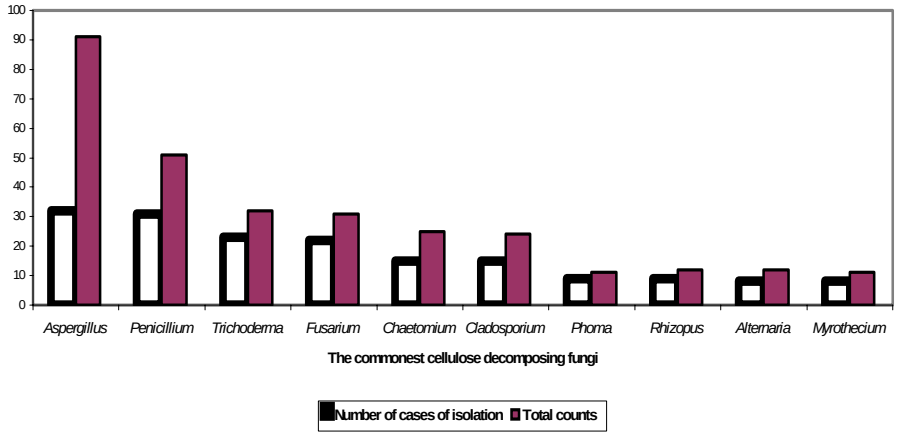

Fig. 2. Total counts (out of 379 isolates) and number of cases of isolation (out of 60 rainfall samples) of the most common terrestrial fungal genera recovered on CCDA medium at 28^{O}C.

Four genera were recovered in moderate frequency of occurrence (14-23 rainfall water samples each). These genera could be arranged descendingly regarding to their number of cases recovered from rainfall water samples (Fig. 2) as follows: *Trichoderma* (3 species, 23 samples, 32 colonies), *Fusarium* (3 species, 22 samples, 31 colonies), *Chaetomium* (3 species, 15 samples, 25 colonies) and *Cladosporium* (2 species, 15 samples, 24 colonies). *Paecilomyces* (*P. variotii*, *P. terricola*, 9 samples, 20 colonies), *Phoma* (*P. glomerata*, 9 samples, 11 colonies), *Rhizopus* (*R. rhizopodiformis*, 9 samples, 12 colonies), *Alternaria* (*A. alternata*, 8 samples, 12 colonies), *Myrothecium* (*M. verrucaria*, 8 samples, 11 colonies), *Botryotrichum* (*B. atrogriseum*, 7 samples, 11 colonies) and *Drechslera* (*D. halodes*, 7 samples, 8 colonies) were occurred and isolated in low frequency of occurrence from the accumulated rainfall water samples. The rest genera of terrestrial fungi were encountered and isolated from rainfall water samples in

rare incidence and these were; *Neurospora* (*N. crassa*, 5 samples, 7 colonies), *Mucor* (*M. circinelloides*, 4 samples, 7 colonies), *Trichothecium* (*T. roseum*, 4 samples, 6 colonies), *Emericella* (*E. nidulans*, *E. quadrilineata*, 3 samples, 7 colonies), *Epicoccum* (*E. nigrum*, 3 samples, 5 colonies), *Fennellia* (*F. flavipes*, 2 samples, 5 colonies) and *Giocladium* (*G. roseum*, 2 samples, 3 colonies).

In accordance with these results, several other authors repeatedly isolated most of these species of terrestrial fungi from various substrata of water, mud and soil in Saudi Arabia [18-20]. These species were also recovered and isolated from other countries; in Syria [4] and in Egypt [7]. Most of the isolated species taxa of terrestrial fungi are well known for cellulose-decomposing fungi as reported by Malik and Eggins [42], Walsh and Stewart [43], Stewart and Walsh [44], El-Nagdy and Nasser [19] and Ali and Nasser [20].

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وجود فطريات التربة في مياه الأمطار المتراكمة في المنطقتين الشرقية والغربية بالمملكة العربية السعودية

ليلى ناصر

كلية التربية للبنات، الرياض، المملكة العربية السعودية

(قدم للنشر في ١٤٢٥/١/٢٥هـ؛ قبل للنشر في ١٤٢٥/١١/٧هـ)

ملخص البحث. تم خلال هذا البحث دراسة وجود فطريات التربة وذلك في ٦٠ عينة من عينات مياه الأمطار المتراكمة والتي تم تجميعها من مناطق مختلفة في المنطقتين الشرقية والغربية بالمملكة العربية السعودية. من هذه العينات تم تعريف وعزل ٤٥ نوعا تنتمي إلى ٢١ جنسا من فطريات التربة وذلك على وسطي العزل الجلوكوز والسيليلوز شابكس دوكس آجار عند ٢٨°م. كان وسط السيليلوز شابكس دوكس آجار (٣٨ نوعا تنتمى إلى ٢٠ جنسا) أغنى بتباين أنواع فطريات التربة عند مقارنته بوسط الجلوكوز شابكس دوكس آجار (٣٥ نوعا تنتمى إلى ١٦ جنسا). كانت معظم أنواع فطريات التربة التي تم عزلها متشابهة على وسطي العزل ومع هذا وجدت بعض الأنواع التي تم عزلها على وسط واحد فقط منهما كان أسبرجيلس وبنسيليوم من أكثر فطريات التربة شيوعا وذلك على كلا وسطي العزل الذين تم إستخدامهما، أيضا كان جنسا أسبرجيلس وبنسيليوم من أغنى أجناس فطريات التربة ثراءا بالأنواع حيث مثلا بعشرة وسبعة أنواع على الترتيب في حين أعطى العديد من الأجناس نوعا واحدا. أظهرت الخصائص الطبيعية والكيميائية للعينات المختبرة من مياه الأمطار المتراكمة عدم وجود دور للرقم الهيدروجيني والمحتوى العضوي في وجود الفطريات بهذه العينات بينما كان تنوع هذه العينات بفطريات التربة يتأثر عكسيا بكمية الأملاح الذائبة بها.