Epidemiologic Study of Opportunistic Fungi Contaminating Soil of Schools, Mosques and Hotels in Diwaniya City - Iraq

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Summary

Soil mycoflora in several schools, mosques and hotels in Diwaniya city were examined using dilution plate technique and three different culture media with and without supplementation of cycloheximide antifungal. A total number of fungal isolates observed in all experiments were 2589 that represent 57 species related to 48 genus, in addition to the white and dark sterile mycelium.

Cycloheximide substantially affected the frequency of isolated fungi. Where *Penicillium chrysogenum* was the most frequent species (44.73%) on culture media free from cycloheximide. *Acremonium kiliense* was the most frequent one (26.85%) on media supplemented with this antifungal. In contrast the frequency and occurrence of *P. chrysogenum* in both soils of school (41.26%) and (95.95%) respectively and of mosques (43.63%) and(100%) respectively was higher than other species, also this species was the most occurring (90%) in hotels soil. *Memoneilla subimplex* was the most frequent species (18.87%) in this soil.

Number of isolates were varied according to the addition of cycloheximide and the composition of culture media: highest number of fungal isolates (337) were recorded when potato dextrose agar used, in contrast the greatest numbers of fungal species (21) were identified when sabouraude dextrose agar was used. In culture media free of cycloheximide the number of fungal isolates and fungal species were substantially increased to 720 and 37 respectively.

The results of this experiment revealed a negative correlation between soil fungi and pH (as r= 0.307 with fungal isolates and 0.185 with the number of fungal species) while this correlation was positive with organic content of soil salinity (r= 0.042 and 0.130 with fungal isolates and fungal species respectively) moreover the results showed negative correlation between fungal isolates and soil salinity (r=0.202) while the number of species of fungal society was positively correlated (r= 0.109).

The hemolytic activity of 30 species in vitro using blood agar culture:18 species showed variable capabilities in hemolysis. *Aspergillus fumigatus* was

with more capability than other examined fungi in tested blood which took 3 days only while this ability of other species was varied from 4 to 12 days .

Introduction

Several epidemiologic studies indicate that children and adult persons who live in environments with high population density such as hospitals, schools, hotels and mosques are exposed to and so liable for infections by opportunistic fungi(1).These environments represent suitable media for fungal growth and diversity(2). In Nigeria,55% of school children were suffering dermatologic fungal infections(3),while in India the prevalence was 13.9%(4).In Nablus,101 fungal species were isolated from scalps of 1389 child in their primary schools(5),while in Egypt the isolated fungal species were 33(6). It was found in Germany that every gram of dust contains 1^{10} - 3.2^{10} CFU(7). .The fungi play a vital role in nature via the damage and recycling of organic matters in soils by secreting enzymes that dissolve the complex compounds in these matters to produce secondary metabolites such as antibiotics and toxins(8). These metabolites are pathogenic for humans and animals(9). These healthy hazards become more prevalent in environments especially in workplaces and crowded areas leading to various diseases mostly in immunocompromised patients(10,11).

Materials & Methods

A total of 151 soil samples (99 school ,32 mosques and 20 Hotels)were collected during the period Jan.2004 to May 2005 in AL-Diwaniya city for isolation and identification of fungi , three types of culture media were used viz; Sabouraud`s Dextrose Agar supplemented with chloramphenicol and cycloheximide and potato Dextrose Agar with chloramphenicol.The dilution method(power-plate technique) was used for growing and isolation of Fungi(14) .After incubation at 29 ºC for 2-4 weeks , cultures were isolated as purified cultures on slant media for preservation in 4 ºC until use for identification and classification of isolated fungi were done depended upon taxonomic literatures(15,16, 17).

To determine the relationship between the community structure of fungi and soil characters such as pH , salinity and organic matter , these parameters were estimated for each soil sample by using the

routine procedure (18,19) . on the other hands , the ability of isolated fungi to hemolysis , 30 species re tested on blood agar in vitro depended on procedure of (20). The analysis of community structure of isolated fungi was done viz , total count of isolates for each genus and species, frequency percentage , occurrence percentage , diversity and dominancy of fungal species for each studied environment(21). Statistical analysis was done using t-test , analysis of variance (ANOVA) table and correlation coefficient for data analysis

Results

A total of 57 fungal species belong to 48 genera in addition to white and black mycelia(table 1).There are 2589 isolates were identified (1900 on media without cycloheximide and 689 on media with cycloheximide)(table 2).

In soil of schools , 2079 isolates were recorded belong to 54 species, while in soils of mosques , 314 isolates were diagnosed belong to 21 species where as in soils of hotels , 196 isolates were collected belong t22 species as in (table 3).

Table 1 : Fungal spies isolated and diagnosed

Gliocladium roseum (Link) Thorn	Absedia cylindrospora Hagem
Gonatorrhodiella sp.	Acremoniella atra (Corda) Sacco
Gonytrichum macrocladum (Sacc.) Hughes	Acremonium kiliense Grutz
Haplographium sp.	Acrogenospora sp.
Haplosporangium sp.	Alternaria alternata (Fr.) Keissler
Humicola grisea Traaen	Ahrinium phaeospermum (Corda)M.B.Ellis
Lacellillvpsis sacchari Subrarn	Aspergillus candidus Link
Memnoniellsubsimplex	A.flavus Link
Monilia sp.	A.fumigatus Fresenius
Monilochaetes sp,	A.niger van Tieghem
Mucor hiemalis Wehrner	Aspergilhls sp.
Paecilomyces marquandii (Massee) Hughes	A.terreus Thorn
Penicillium. chrysugenum Thom	A.ustus (Bainier) Thorn & Church
Perlcoma byssoidesPers.ex Me'rat .	Aureobasidium pullulans (deBary) Arnaud
Pithomyces maydicu.5 (Sacc.) M.B, Ellis	astomyces dermatitidis Gilchrist & Stokes
Plieochaet setosa(Kirchn.) Hughes	Cephaliophora irregularis Thaxter
Rhizoclonia solani Kuhn	Chaetomium aurum Chivers
Rhizopus stolonifer (Ehrenb.ex Fries) Lind	Chaetophoma sp,
.Scytalidiuln lignicola Pesante	Chrysosporium keratinophilum (Frey) Carmichael
.Sporolhrix schenckii Hekton & Perkins	Cladosporium cladosporioides (Fresen) de Vries
,Staphylotrichum coccosporum Meyer & Nicot	Cochlonema sp
.Stemphylium sarciniforme (Cav.) Wiltshire	Dark mycelia
Streptomyces sp .	Doratomyces stemonitis (Pers.ex Fr.) Morton & Smith
Trichobotrys effuse Berk. & Br.) Petch	Drechslera australiensis(Bugnicourt)Subram & Jain ex M.B. Ellis
Trichoderma harziamlm Rifai	D.dematioidea (Bubak & Wrblewski) Subram& Jain.
T. viride Pers. ex Gray	D.siccans (Drechsler) Shoemaker
Ulocladium bouytis Preuss	Eurotium sp
Verticillium lateritillm Berk	Fusarium moniliforme Scheldon
White mycelia	Gilmaniella sp.
	Gliocephalolrichum sp,

Table (2) Total number of fungal isolates from study Environments on media with or without cycloheximide

Species	No.of isolates					
	CDA		PDA		SDA	
	Y	-CY	+CY	-CY	+CY	-CY
bsedia cylindrospora	2	10	-	1	1	4
Acremoniella atra	-	-	6	-	13	-
Acremonium kilielrse	71	104	65	127	49	74
Acrogenospora sp	-	-	-	1	-	-
Alternaria alternata	3	18	3	17	3	12
Artlrrinium plraeospermum	-	-	-	1	-	-
Aspergillus candidus	-	4	1	-	1	10
Aflavus	-	3	12	18	-	5
A.fumigatus	1	8	-	-	-	26
niger	1	633	-	31	-	2
Aspergillus sp.	1	46	-	-	3	3
A.terreus	29	26	43	17	22	27
A.uus	1	4	-	5	1	-
Aureobasidium pullulans	-	-	-	-	-	1
Blastomyces dermatitidis	-	1	-	-9	-	-
Cephaliophora irregularis	-	31	-	-	-	-
Chaetomium aurum	-	3	-	3	-	3
Chaetophoma sp.	-	01	-	-	-	-
Chrysosporium keratinoplrilum	-	1	7	-	77	7
Cladosporulm cladosrioides	6	62	8	25	1	49
Cochlema sp.	1	7	-	81	-	2
Dark mycelia	-	6	-	2	-	9
Doratomyces stemonitis	4	-	-	-	-	-
Drechslera australiensis	-	1	-	-	-	1
D.dematioidea	-	2	-	-	-	1
D.siccans	-	30	-	232	9	1
Eurotium sp.	-	-	-	-	-	-
Fusarium moniliforme	-	-	-	1	-	3
Gilmaniella sp.	-	-	-	1	-	-
cephalotrichum sp.	-	-	-	1	-	-
Gliocladium roseum	-	15	-	1	-	-

In soil of school , 2079 isolates were recorded belong to 54 species , while in soils of mosques , 314 isolates were diagnosed belong to 21 species where an in soils of hotels , 196 isolates were collected belong to 22 species as in(table 3)

Table (3) Total Number of isolates and number of species according to study Environment and media used

Environment	media	CDA		PDA		SDA	
		+CY	-CY	+CY	+CY	-CY	+CY
Mosques	Total isolates	29	2113	432	277	26	26
	Species No.	3	5	4	8	6	7
Hotels	Total isolates	15	38	71	10	31	31
	Species No.	8	9	11	5	7	9
Schools	Total isolates	134	69	23	83	17	453
	Species No.	13	36	12	32	16	29

The data of fungal community structure analysis revealed that the penicillium chrysogenum was most frequent and occuente in comparism with other isolated fungi in all studied area (table 4, 5,6)

Table (4) Dominance and percentage of frequency and occurrence of isolated fungi in Schools

species	frequency%	occurrence %	Dominance type
Absedia cylindrospora	0.38	27.27	R
Acremoniellaatra	0.14	1.01	R
Acremonium kilielrse	22.84	63.63	D
Acrogenosora sp	0.04	1.01	R
Alternaria alternata	2.21	75.75	SD
Artlrriniumplraeospermum	0.4	01	R
Aspergillus candidus	072	2727	R
Aflavus	1.05	2.27	C
A.fumigatus	0.81	31.31	C
A.niger	1.29	59.59	C
Aspergillus sp.	0.38	23.23	R
A.terreus	2.74	67.67	SD
A.ustus	0.43	19.19	R
Aureobasidium pullulans	0.04	1.01	R
Blastomyces dermatitidis	0.04	1.01	R
Cephaliophora irregularis	0.04	1.01	R
Chaetomium aurum	0.33	19.19	R
Chaetophoma sp.	0.04	1.01	R
Chrysosporium keratinoplrilum	1.05	23.23	C
Cladosporulmcladosporioides	7.21	79.79	SD
Cochlonema sp.	0.52	19.19	R
Dark mycelia	076	7.7	C
Doratomyces stemonitis	0.19	2.02	R
Drechslera australiensis	0.09	202	R
D.dematioidea	0.14	3.03	R
D.siccans	0.28	4.04	R
Eurotium sp.	0.43	1.01	R
Fusarium moniliforme	0.19	4.04	R
Gilmaniella sp.	0.04	1.01	R
Gliocephalotrichum sp.	0.04	1.01	R

Table(5)Dominance and percentage of frequency and occurrence of isolated fungi in hotels

Absedia cylindrospora	2.04	15	R
Acremoniella atra	7.14	35	SD
Acremonium kilielrse	7.65	275	SD
Alternaria alternata	3.06	5	R
Aspergilhls flavus	8.816	65	SD
A.fumigatus	2.55	225	R
A.niger	7.65	65	SD
Aspergillus sp.	18.02	5	R
A.terreus	6.63	55	SD
A.ustus	0.51	25	R
Chaetomium aurum	521.02	25	R
Haplographium sp	0.51	5	R
Memnoniella subsimplex	18.87	30	D
Mucor hiemalis	0.51	5	R
Paecilomyces marquandii	1.02	10	R
Pellicillium chrysogellum	16.32	90	D
Periconia hyssoides	3.57	0	C
Rhizopus stolonifer	1.02	25	R
Scytalidium ligllicola	02.51	5	R
Sporothrix schenckii	1.02	2310	R
Streptomyce sp.	2.04	20	R
Verticillium lateritium	2.55	15	R
White mycelia	5.10	40	C

Statistical analysis showed a significant difference ($P< 0.05$) between frequency and occurrence of fungi cultured on media with or without cycloheximide .the other fungi varied in their frequency and occurrence according to study area and type of media used In general the P.chryogenum and aspergillus terreus were the dominate in soils of the study area .on the other hands , results revealed that the schools environment was the most diversity in fungal community rather than masques and hotels area

Table (6) : Values of soil factors in samples from study environments

Environment	Organic mggatter%	Salifvgffnity %	pH
Schools	0.09 ± 0.46	0.9ggg9 ± 2.61	0.94 ± 7.35
Mosques	010 ±11 0.48	0.73 ± 2.12	110.79 ±7.47
Hotels	0.09 ± 0.53	0.62 ± 2.59	0.12 ± 6.85

The estimation of correlation coefficient (r) for soil charcters and fungal community structure a negative relationship between pnnbvnH anderer salinity aeernd positive for organic matter with community structure (fig. 1,2,3)

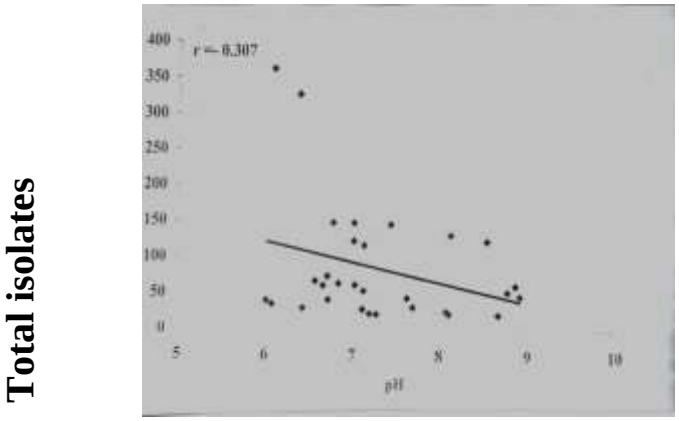

Figure (1) Relation ship between pH Values in Soils and total isolates

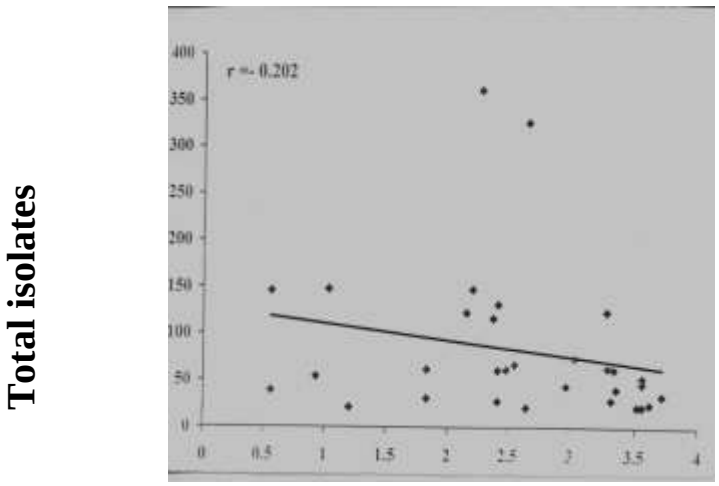

Figure (2) Relationship between salinity percent in Soils and total isolates

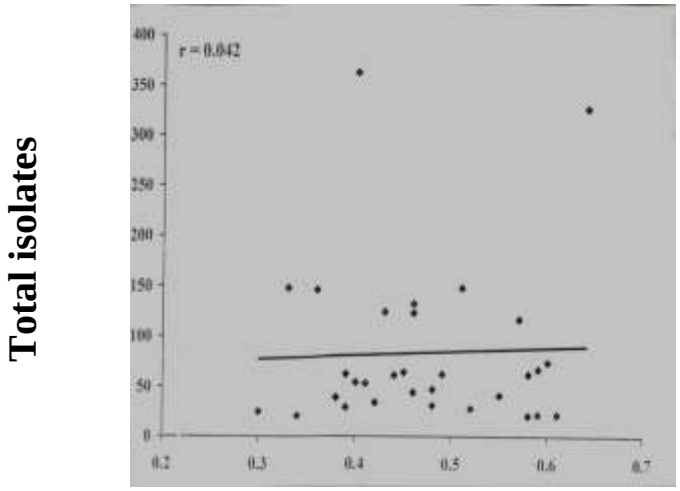

Figure (3) Relationship between organic matter percent in Soils and total isolates

Table (7) shows the results of haemolysis for 30 species which tested in vitro on blood agar plates , where only 18 species were capable to hemolysis with avariations in diameter of lasis zone

and time required for lysis . The Aspergillus famigates was the quicker in haemolysis in comparison with others

Discussion

The data revealed that the type of technique used in isolation and type of culture media with or without cyclohximida play a vital role in the frequency ,occurrence and dominance of fungi , this may be due to the effect of media composition and assimilation or adsorption and metabolism of dissolved material in media .this may explain the domminan of P.chrysogenum in all sturdiest area which is established by other studies (22,23,24,25,26).

The reasons of this dominancy may due to it's a high productivity of small spores in the environments , in addition to ability to grow in a wide range of temperature (4 – 45)C (27).

Species	Diameter of Haemolysis (cm)	Timeof haemolysis(days)
Acrogenospora sp.	1.1	6
Aspergillus fumigatus	23.3	3
Blastomycedermatitidis	2.555	55558
Chrysosrium keratinophilum	3.3	5
Cochloma sp.	*3.3*	*8*
Doratomyces stemonitis	*3.3*	*4*
Eurotium sp.	*3.755*	*8*
Gonytrium macrocladum	*52.7*	*6*
Haplographium sp.	*3.0*	*6*
Haplosporangium sp.	*4.9*	*8*
Huma grisea	*1.0*	
Lacellinopsis sacchari	*3.9*	*8*
Memnoiella subsimplex	*3.2*	*8*
Monilochaetes sp.	*3.9*	*5*
Rhizoctonia solani	*3.9*	*8*
Sporothrix schenckii	*3.552*	*8*
Staphylotrichum coccosporum	*1.4*	*12*
Verticillium lateritium	*2.7*	*5*

Also , the study show that the school environment was the most diversity in fungal community in comparison with other studied environments . This may be related to the percentage of organic

matter in soils of the schools rather than hotels or mosques (28) . On the other hands, the prevalence of keratinophylic and opportunistic fungi in the sgfhgoils of studied area may be due to the presence of keratin materials in these environments that leads to the increased distribution of these fungiand so to an ihththyncreased probability of its pathogenicity to humans (29,30).

Regarding the effect of soli factors on variety and volume of fungal community , results revealed fungal preference to acidic PH(5-6), This may aid these fungi in lowering their competition wwith bacteria, actinomycetes and saprophetic fungi in acidic environ756u7675676ment (31). This is in agreement with other studies (32,33). Other studies indicated this relationship between the salinity and organic matter with community structure of fungi (34,35). This may explain the negative and positive correlation cuu6uoeffiecient between these factors and frequency or occurrence of some fungi that tolerate salinity (36,37).

The ability of some fungi to hemolyes in vitro may explain the presence of factors of virulence such as enzymes or toxins capable to damage the tissues and causes various diseases to humans(38).

Conclusions and recommendations:

The environments in studied areas contain higher number of fungal species with higher diversity . The highest of which is in school environment, while it is the lowest in hotels . so we recommend higheu76567r attention to be paid in order to limit this fungal contamination.

Most of the opportunistic fungi revealed resistance to cyclohexamide which is known as fungicidal agent. This means t6u767hat they have more ability to infect people especially those with low immunity .

This needs more elaboration by researches on molecular basis for these fungi .

Soil factors such as organic matter, salinity and pH may play a vital role in the frequency, occurrence and dominance of the isolated fungi Investigation of such factors in soils used for establishment of

schools , Mosques and hotels ijyuyjuyjus recommended.

Most of the isolated fungi revealed high ability to lyse the blood . This means that they become more virulent wyujtyuhen infecting humans. This needs more elaborated methods of sterilization to prevent fungal infection.

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