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Mycoflora Isolated from Mazot and Solar Polluted Soils in Upper Egypt

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FORTY-NINE species and two varieties belonging to 22 genera were recovered on dextrose and 1% crude oil Czapek's agar media from 48 soil samples polluted with mazot and solar. The hydrocarbons polluted soils were collected from three governorates in Upper Egypt; namely El-Minya, Assiut and Sohag (8 samples from each governorate for each hydrocarbon). The most common genus was *Aspergillus* which was isolated from the three governorates and from both hydrocarbon polluted soils, the most common species was *Aspergillus fumigatus* isolated from mazot polluted soils, while *Aspergillus flavus, Aspergillus fumigatus* and *Aspergillus terreus* were the most common species isolated from solar polluted soils. Forty fungal isolates belonging to the 22 genera were tested for their ability to utilize crude oil in Czapek's medium at 28 ±1ºC. Out of these fungi, 32 isolates were able to grow forming visual growth and dry mass determined.

One of the key enzymes in oil utilization is lipase, therefore the 40 isolates were subjected to lipase activity test. Remarkably, out of the tested fungi, 35 isolates produced visual growth and lipase activity, while 4 showed growth without producing the enzyme, these were *Aspergillus awamorii, Chrysosporium tropicum, Trichoderma harzianum* and *Ulocladium chartarum*. The highest recorded lipase productivity was observed by *Fusarium verticilloides* isolated from solar polluted soil at El-Minya giving 12.28 U/ml.

Environmental pollution is a worldwide problem and its potential to influence the health of human populations is great (Fereidoun *et al*., 2007). Hydrocarbon pollution is a serious problem in the environment and represents 70% of environmental pollutants (Lazar *et al*., 1995a,b). Petroleum hydrocarbons are the most common types in the environment. Petroleum-based products are the major source of energy for industry and daily life, thus they can cause serious pollution problems in both the aquatic and the terrestrial environments (Plohl *et al.*, 2002).

Soil pollution with hydrocarbons causes extensive damage of local environmental system since accumulation of pollutants in animal and plant tissues may cause death or mutations (Alvarez and Vogel, 1991). Petroleum hydrocarbons in soils adversely affect the germination and growth of plants in soils by creating conditions which make essential nutrients like nitrogen and oxygen needed for plant growth unavailable to them. This effect could be produced by oily hydrocarbons which acts as a physical barrier preventing or reducing access of the seeds to water and oxygen (Ogbo *et al.*, 2010). Hydrocarbon pollution can cause human cancers, including leukaemia and increase lead concentration in soil which is especially hazardous for young children causing developmental damage to the brain (Collins, 1998).

The technologies commonly used for soil remediation from hydrocarbon pollutants include: mechanical burying, evaporation, dispersion, and washing. However, these technologies are expensive and can lead to incomplete decomposition of contaminants. Among the technologies now available and has more advantages to deal with polluted soils are bioremediations; based on the metabolic activity of microorganisms for degradation of hydrocarbons (Van Gestel *et al*., 2003).

The use of fungi in bioremediation has received considerable attention for their potent bioremediation, by producing enzymes involved in degradation process of wide range of hydrocarbons (Husaini *et al*., 2008).

The aim of the present study is to survey the mycoflora found in polluted soils from three governorates in Upper Egypt (El-Minya, Assiut and Sohag) polluted with petroleum hydrocarbons (mazot and solar). Production of lipase by the isolated fungi was also investigated.

Materials and Methods

Samples collection

Forty-eight soil samples were collected from three governorates in Upper Egypt (El-Minya, Assuit and Sohag) of which 24 samples were mazot polluted and the other 24 samples were solar polluted soils. Samples were taken at a depth of 5 inches from surface with auger and directly placed in clean sterilized polyethylene bags, transferred to the laboratory and kept at 4˚C until use.

Media used in this study

Two media were used for isolation of crude oil decomposing fungi: 1% dextrose Czapek's agar medium g/liter (agar, 15.0; $NaNO_3$, 2.0; KH_2PO_4, 1.0; $MgSO_4.7H_2O$, 0.5; KCl, 0.5; $FeSO_4.7H_2O$, 0.01; glucose, 10.0; distilled water, 1000 ml), and 1% crude oil Czapek's agar medium in which the 10 g dextrose were replaced by 10 ml crude oil. Rose Bengal (1/30000) and chloramphenicol, 250 mg/L were added to the medium as bacteriostatic and bactericidal agents, respectively (Smith and Dawson, 1944). Twelve plates (6 plates for each type of medium) were used by adding media to1 ml aliquots of 1/100 soil suspension in sterile Petri dishes. The plates were incubated at 28 ±1ºC for 7-9 days and the developing fungi were identified and counted.

Screening for crude oil utilizing fungi

Liquid crude oil Czapek's medium was used for testing crude oil utilization of the isolated fungi. Spore suspension was prepared for each isolate. One ml of the spore suspension was inoculated to 30 ml of 1% crude oil Czapek's medium in 100 ml volume Erlenmeyer flasks and the flasks were incubated at 30 ± 1˚C on shaker (150 rpm) for two weeks. Subsequently, the dry mass of fungal mat was determined.

Screening for extracellular lipase production

Modified basal salt medium (BSM) containing (g/l): NH_4NO_3, 1; K_2HPO4, 1.5; $MgSO_4.7H_2O$, 0.025; $CaCL_2$, 0.025; $FeSO_4.7H_2O$, 0.015; $ZnSO_4.7H_2O$, 0.005, distilled water, 1000 ml and pH adjust to 7. After sterilization of medium chloramphenicol, 250 mg and 1% (v/v) sterilized tween 80 were added (Nakajima-Kambe *et al.,* 1999 and Sahin *et al.* 2000). One ml of the spore suspension was transferred to 30 ml of pervious medium. Inoculated flasks were incubated at 30 ± 1˚C under shaking (150 rpm) for 6 days, then filtrate was centrifuged under cooling at 10000 xg for 30 min at 4^0C, and the supernatants was subsequently collected for lipase activity determination. Lipase activity was determined by using p-nitrophenylpalmitate (pNPP) as described by Licia *et al.* (2006).

Results

Fungi isolated from mazot polluted soils

Results presented in Table 1 indicate that 18 species belonging to 8 genera were isolated from 24 mazot polluted soil on 1% dextrose Czapek's agar medium. The total counts of fungi isolated from 24 mazot polluted soils were 6832.5cfu/g. Polluted soils from El-Minya governorate showed the highest fungal population presenting 36.54% of the total fungi counts, whereas the total counts in Assiut and Sohag governorates presented 35.57% and 27.88% of the total isolated fungi, respectively.

Remarkably, *Aspergillus* was the most dominant genus which emerged in high frequency yielding 75.48% of total fungi, whereas the genus *Penicillium* came in second occurrence rank giving rise to 12.5% of total fungi, and the genus *Cladosporium* came in third occurrence rank contributing 3.845% of total fungi. Whereas the genera; *Fusarium, Eurotium, Alternaria, Stachybotrys* and *Chaetomium* were of rare frequency occurrence, represented by one species for each of them namely, *F. verticilloides, E. repens , A. alternata, S. chartarum* and *C. spirale*, accounting for 2.885%, 1.44%, 0.96%, 0.96% and 0.48% of total fungi, respectively on 1% dextrose Czapek's agar medium.

Eleven species belonging to 5 genera were isolated from the 24 mazot polluted soil on 1% crude oil Czapek's agar medium, (Table 2). The total counts of fungi using this crude oil medium were 6166.5cfu/g; represented by 57.29% of this total fungi from Sohag and by 24.32% and 18.37%, respectively from El-Minya and Assiut governorates .

TABLE 1. Total counts (calculated per gm soil), percentage total counts (calculated per total fungi), number of cases of isolation (out of 24 samples) and occurrence remarks of fungi isolated from mazot polluted soils collected from three governorates on 1% dextrose Czapek's agar medium at 28±1°C.

Governorate	El-Minya				Assiut				Sohag				Total			
Fungal species	TC	%TC	NCI	OR	TC	%TC	NCI	OR	TC	%TC	NCI	OR	TC	%TC	NCI	OR
Aspergillus	1566.5	61.84	7	H	1933.2	78.38	8	H	1733.1	94.55	8	H	5232.8	76.58	23	H
Alternaria alternata	0	0	0		66.6	2.7	1	L	0	0	0	0	66.6	0.97	1	R
Aspergillus awamorii	0	0	0	0	0	0	0	0	100	5.45	1	L	100	1.46	1	R
Aspergillus flavus	200	7.89	2	L	500	20.27	5	H	466.6	25.45	4	M	1166.6	17.07	11	M
Aspergillus fumigatus	1199.9	47.37	6	H	600	24.33	6	H	1166.5	63.64	8	H	2966.4	43.45	20	H
Aspergillus niger	0	0	0	0	266.6	10.81	2	L	0	0	0	0	266.6	3.9	2	R
Aspergillus ochraceus	0	0	0	0	100	4.05	1	L	0	0	0	0	100	1.46	1	R
Aspergillus oryzae	0	0	0	0	100	4.05	1	L	0	0	0	0	100	1.46	1	R
Aspergillus sydowii	166.6	6.57	2	L	0	0	0	0	0	0	0	0	166.6	2.44	2	R
Aspergillus terreus	0	0	0	0	300	12.16	2	L	0	0	0	0	300	4.39	2	R
Aspergillus ustus	0	0	0	0	66.6	2.7	1	L	0	0	0	0	66.6	0.97	1	R
Chaetomium spirale	0	0	0	0	0	0	0	0	33.3	1.85	1	L	33.3	0.48	1	R
Cladosporium	100	3.95	1	L	166.6	6.75	1	L	0	0	0	0	266.6	3.9	2	R
Cladosporium cladosporioides	0	0	0	0	166.6	6.75	1	L	0	0	0	0	166.6	2.44	1	R
Cladosporium herbarium	100	3.95	1	L	0	0	0	0	0	0	0	0	100	1.46	1	R
Eurotium repens	0	0	0	0	100	4.05	1	L	0	0	0	0	100	1.46	1	R
Fusarium verticilloides	0	0	0	0	200	8.11	1	L	0	0	0	0	200	2.93	1	R
Penicillium	866.6	34.21105	3	M	0	0	0	0	0	0	0	0	866.6	12.6835	3	L
Penicillium cyclopium	466.6	18.42	2	L	0	0	0	0	0	0	0	0	466.6	6.83	2	R
Penicillium puberulum	400	15.79	1	L	0	0	0	0	0	0	0	0	400	5.85	1	R
Stachybotrys chartarum	0	0	0	0	0	0	0	0	66.6	3.63	1	L	66.6	0.97	1	R
Total count	2533.1	100			2466.4	100			1833	100			6832.5	100		
No. of genera	3				5				3				8			
No. of species & varieties	6				11				5				18			

TC = Total counts, %TC = Percentage of total counts, NCI = Number of cases of isolation, OR = Occurrence remarks.
H= High occurrence; more than 12 samples out of 24 samples, M= Moderate occurrence; between 6-11 samples, L= Low occurrence; between 3-5 samples, R= Rare occurrence; less than 3 samples.

TABLE 2. Total counts (calculated per gm soil), percentage total counts (calculated per total fungi), number of cases of isolation (out of 24 samples) and occurrence remarks of hydrocarbon degrading fungi isolated from mazot polluted soils collected from three governorates on 1% crude oil Czapek's agar medium at 28±1°C.

Governorate	El-Minya				Assiut				Sohag				Total			
Fungal species	TC	%TC	NCI	OR	TC	%TC	NCI	OR	TC	%TC	NCI	OR	TC	%TC	NCI	OR
Alternaria alternata	0	0	0	0	200	17.65	2	L	0	0	0	0	200	3.21	2	R
Aspergillus	1500	95.75	6	H	566.6	50	5	H	3333.3	94.34	8	H	5399.9	86.63	19	H
Aspergillus awamorii	0	0	0	0	0	0	0	0	100	2.83	1	L	100	1.6	1	R
Aspergillus flavus	200	12.76	1	L	0	0	0	0	300	8.49	2	L	500	8.02	3	L
Aspergillus fumigatus	1200	76.59	5	H	366.6	32.35	3	M	700	19.81	3	M	2266.6	36.36	11	M
Aspergillus melleus	0	0	0	0	0	0	0	0	33.3	0.94	1	L	33.3	0.53	1	R
Aspergillus niger	100	6.38	1	L	100	8.82	1	L	500	14.15	1	L	700	11.23	3	L
Aspergillus terreus	0	0	0	0	100	8.82	1	L	1600	45.28	3	M	1700	27.27	4	L
Aspergillus versicolor	0	0	0	0	0	0	0	0	100	2.83	1	L	100	1.6	1	R
Chrysosporium tropicum	0	0	0	0	300	26.47	2	L	0	0	0	0	300	4.8	2	R
Penicillium cyclopium	66.6	4.25	2	L	0	0	0	0	0	0	0	0	66.6	1.07	2	R
Stachybotrys chartarum	0	0	0	0	0	0	0	0	200	5.66	2	L	200	3.21	2	R
Trichoderma harzianum	0	0	0	0	66.6	5.87	1	L	0	0	0	0	66.6	1.07	1	R
Total count	1566.6	100			1133.2	100			3533.3	100			6233.1	100		
No. of genera	2				4				2				6			
No. of species & varieties	4				6				8				12			

TC = Total counts, %TC = Percentage of total counts, NCI = Number of cases of isolation, OR = Occurrence remarks.

H= High occurrence; more than 12 samples out of 24 samples, M= Moderate occurrence; between 6-11 samples, L= Low occurrence; between 3-5 samples, R= Rare occurrence; less than 3 samples.

Also, on this crude oil medium, among the genera recorded, *Aspergillus* was the most dominant genus which was isolated in high frequency yielding 87.568% of total fungi. The genera *Alternaria, Chrysosporium, Stachybotrys* and *Trichoderma* were isolated in rare frequency giving rise to 3.24%, 4.86%, 3.24% and 0.632% of total fungi count.

Fungi isolated from solar polluted soils

Thirty four species in addition to two varieties belonging to 16 genera were isolated from 24 solar polluted soils on 1% dextrose Czapek's agar medium (Table 3). The total counts were 24696.7cfu/g. Soil samples collected from Sohag governorate showed the highest fungal population accounting for 42.64% of the total fungi, while the total fungal counts in soil samples from El-Minya and Assiut governorates, respectively presented 35.89% and 21.45% of total isolated fungi.

Generally, the genus *Aspergillus* was the most dominant genus in solar polluted soils yielding 75.31% of total fungi counts, while the genus *Penicillium* came in second rank, recording 7.69% of total fungi. The genera *Aureobasidium, Phoma, Absidia, Acremonium, Alternaria, Cunninghamella, Cochliobolus, Rhizopus* and *Syncephalastrum* were represented by one species for each of them, namely, *P. lilacinus, C. cladosporioides, A. pullulans, P. herbarium, A. cylindrospora, A. strictum, A. alternata, C. echinulata, C. specifer, R. Oryzae,* and *S. racemosum*. Their rare counting frequency comprised 1.35%, 0.27%, 0.135%, 0.135%, 0.135%, 0.135%, 0.135%, 0.135% and 0.135% of total fungi, respectively on 1% dextrose Czapek's agar medium.

Data in Table 4 show that on 1% crude oil Czapek's agar medium, 17 species belonging to 8 genera were isolated from the 24 solar polluted soil samples. The total fungal counts were 20199.2cfu/g. El-Minya governorates contributed the highest fungal populations (45.09% of the total fungi), while Sohag governorate contributed the lowest fungal populations (25.49% of the total counts).

Also, similar to the results obtained on dextrose Czapek's agar medium, the genus *Aspergillus* was the most prevalent on 1% crude oil Czapek's agar medium, isolated in high frequency yielding 92.59% of total of fungi, while the genus *Penicillium* came on second rank, contributing 1.12% of the total fungi, and the genus *Rhizopus* ranked in third place accounting for 0.64% of the total fungi. But, the genera *Absidia, Chrysosporium, Fusarium, Mucor* and *Ulocladium* were isolated in rare frequency on this medium.

Screening of fungal isolates for crude oil utilization

Table 5 shows that out of the forty tested fungi screened for crude oil utilization, fourteen fungal isolates belonging to genera *Absidia, Aspergillus, Cochliobolus, Fusarium* and *Stachybotrys* have been recorded as high crude oil utilizers, giving fungal dry mass higher than 60 mg/30 ml culture. Another 6 isolates belonging to *Aspergillus* and *Penicillium* were recorded as moderate crude oil utilizers, giving fungal dry mass between 30-59 mg/30 ml culture, and 12 fungal isolates as low crude oil utilizing fungi, giving dry mass less than 30 mg/30 ml culture. Eight fungal isolates didn't give any visual growth on liquid 1% crude oil Czapek's medium. These included one isolate of each of *Aspergillus awamorii, Chrysosporium tropicum, Rhizopus stolonifer, Syncephalastrum racemosum* and two isolates of *Stachybotrys chartarum* and *Ulocladium chartarum.*

Screening of fungal isolates for lipase production

Out of the forty fungal isolates screened for lipase production, eleven isolates belonging to the species *Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus terreus, Cochliobolus specifer, Fusarium verticilloides* and *Penicillium chrysogenum* were recorded as high lipase producer (Table 6). The most active isolate was *Fusarium verticilloides* giving 12.28 U/ml. .

Eight fungal isolates belonging to the species *Absidia cylindrospora*, *Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus terreus*, *Fusarium oxysporum* and *Fusarium solani* were recorded as moderate lipase producer and twelve fungal isolates as low lipase producer. The lowest activity was shown by *Chrysosporium keratinophilum* producing 0.679 U/ml. Also, five fungal isolates didn't give any visual growth on lipase production medium, these were: one isolate of *Aspergillus versicolor, Rhizopus stolonifer, Syncephalastrum racemosum, Trichoderma harzianum* and *Ulocladium chartarum.*

Discussion

From the results obtained, we notice that the most common genera isolated in this study were *Aspergillus* and *Penicillium*, while the most common species were *Aspergillus awamorii*, *Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus niger* and *Aspergillus terreus* which were isolated from the two hydrocarbons contaminated soils (mazot and solar) on the two types of media (dextrose – crude oil Czapek's agar) that were used. In agreement with our results, the ability of *Aspergillus* in utilizing petroleum hydrocarbons especially crude oil has been demonstrated by Bartha and Atlas (1977) in aquatic oil spills and Radwan *et al*. (1995) in polluted Kuwait desert.

Also, in accordance with our results, *Aspergillus* species have been reported to be recovered from Egyptian soils treated with aromatic hydrocarbons. Bagy *et al.* (1992) reported that *Aspergillus* and *Scopulariopsis* were the most common genera that have been isolated from oil polluted water. Also, Hemida *et al.* (1993) isolated thirty fungal species and one variety belonging to 14 genera from Egyptian soils treated with petroleum derivatives (mazot and solar), and reported that *Amorphotheca resinae, Aspergillus flavus, Aspergillus fumigatus, Aspergillus niger, Aspergillus terreus, Rhizopus stolonifer and Trichoderma harzianum* were the most common species.

TABLE 3. Total counts (calculated per gm soil), percentage total counts (calculated per total fungi), number of cases of isolation (out of 24 samples) and occurrence remarks of fungi isolated from solar polluted soils collected from three governorates on 1% dextrose Czapek's agar medium at 28 ±1°C.

Governorate	El-Minya				Assiut				Sohag				Total			
Fungal species	TC	%TC	NCI	OR	TC	%TC	NCI	OR	TC	%TC	NCI	OR	TC	%TC	NCI	OR
Absidia cylindrospora	0	0	0	0	0	0	0	0	33.3	0.32	1	L	33.3	0.13	1	R
Acremonium strictum	0	0	0	0	33.3	0.63	1	L	0	0	0	0	33.3	0.13	1	R
Alternaria alternata	33.3	0.37	1	L	0	0	0	0	0	0	0	0	33.3	0.13	1	R
Aspergillus	8299.2	93.61	8	H	2966.2	55.97	8	H	7332.7	69.62	8	H	18598.1	75.31	24	H
Aspergillus awamorii	100	1.13	1	L	166.5	3.14	3	M	666.6	6.33	3	M	933.1	3.77	7	M
Aspergillus clavatus	0	0	0	0	0	0	0	0	33.3	0.32	1	L	33.3	0.13	1	R
Aspergillus flavus	499.9	5.64	4	M	400	7.55	4	M	566.5	5.38	5	H	1466.4	5.94	13	H
Aspergillus fumigatus	1599.8	18.04	8	H	1333.2	25.16	6	H	299.9	2.85	3	M	3232.9	13.09	17	H
Aspergillus niger	199.9	2.25	2	L	166.6	3.14	2	L	0	0	0	0	366.5	1.48	4	L
Aspergillus ochraceus	33.3	0.37	1	L	0	0	0	0	0	0	0	0	33.3	0.13	1	R
Aspergillus oryzae	0	0	0	0	0	0	0	0	33.3	0.32	1	L	33.3	0.13	1	R
Aspergillus sydowii	1066.6	12.03	2	L	0	0	0	0	1133.3	10.76	3	M	2199.9	8.91	5	L
Aspergillus tamarii	33.3	0.37	1	L	0	0	0	0	0	0	0	0	33.3	0.13	1	R
Aspergillus terreus	4066.5	45.86	6	H	899.9	16.98	3	M	4466.5	42.41	8	H	9432.9	38.19	17	H
Aspergillus ustus	699.9	7.89	2	L	0	0	0	0	33.3	0.32	1	L	733.2	2.97	3	L
Aspergillus versicolor	0	0	0	0	0	0	0	0	100	0.95	1	L	100	0.4	1	R
Aureobasidium pullulans	0	0	0	0	0	0	0	0	333.3	3.16	1	L	333.3	1.35	1	R
Cladosporium cladosporioides	0	0	0	0	466.6	8.8	1	L	533.1	5.06	6	H	999.7	4.05	7	M
Cochliobolus specifer	0	0	0	0	0	0	0	0	33.3	0.32	1	L	33.3	0.13	1	R
Cunninghamella echinulata	33.3	0.37	1	L	0	0	0	0	0	0	0	0	33.3	0.13	1	R
Emericella	66.6	0.75	1	L	66.6	1.26	1	0	0	0	0	0	133.2	0.54	2	R
Emericella nidulans var. *lata*	0	0	0	0	66.6	1.26	1	L	0	0	0	0	66.6	0.27	1	R
Emericella nidulans var. *nidulans*	66.6	0.75	1	L	0	0	0	0	0	0	0	0	66.6	0.27	1	R
Fusarium	33.3	0.37	1	L	66.6	1.26	1	L	0	0	0	0	99.9	0.4	2	R
Fusarium nygamia	0	0	0	0	66.6	1.26	1	L	0	0	0	0	66.6	0.27	1	R
Fusarium verticilloides	33.3	0.37	1	L	0	0	0	0	0	0	0	0	33.3	0.13	1	R
Paecilomyces lilacinus	0	0	0	0	1399.9	26.42	2	L	633.3	6.01	1	L	2033.2	8.23	3	L
Penicillium	100	1.13	1	L	299.9	5.66	2	L	1499.8	14.24	5	H	1899.7	7.69	8	M
Penicillium brevicompactum	0	0	0	0	0	0	0	0	66.6	0.63	1	L	66.6	0.27	1	R
Penicillium canscens	100	1.13	1	L	0	0	0	0	0	0	0	0	100	0.4	1	R

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TABLE 3. Continued.

Governorate	El-Minya				Assiut				Sohag				Total			
Fungal species	TC	%TC	NCI	OR	TC	%TC	NCI	OR	TC	%TC	NCI	OR	TC	%TC	NCI	OR
Penicillium chrysogenum	0	0	0	0	0	0	0	0	1333.3	12.66	3	M	1333.3	5.39	3	L
Penicillium citrinum	0	0	0	0	33.3	0.63	1	L	0	0	0	0	33.3	0.13	1	R
Penicillium cyclopium	0	0	0	0	0	0	0	0	33.3	0.32	1	L	33.3	0.13	1	R
Penicillium duclauxii	0	0	0	0	200	3.77	1	L	0	0	0	0	200	0.81	1	R
Penicillium funiculosum	0	0	0	0	0	0	0	0	33.3	0.32	1	L	33.3	0.13	1	R
Penicillium oxalicum	0	0	0	0	66.6	1.26	1	L	33.3	0.32	1	L	99.9	0.4	2	R
Phoma herbarium	66.6	0.75	1	L	0	0	0	0	0	0	0	0	66.6	0.27	1	R
Rhizopus oryzae	0	0	0	0	0	0	0	0	33.3	0.32	1	L	33.3	0.13	1	R
Syncephalastrum racemosum	0	0	0	0	0	0	0	0	33.3	0.312	1	L	33.3	0.13	1	R
Ulocladium	233.3	2.63	2	L	0	0	0	0	66.6	0.63	1	L	299.9	1.21	3	L
Ulocladium constrile	100	1.13	1	L	0	0	0	0	66.6	0.63	1	L	166.6	0.67	2	R
Ulocladium chartarum	133.3	1.5	1	L	0	0	0	0	0	0	0	0	133.3	0.54	1	R
Total count	8865.6	100			5299.1	100			10532	100			24696.7	100		
No. of genera	8				7				10				16			
No. of species & varieties	16+1				12+1				22				35+2			

TC = Total counts, %TC = Percentage of total counts, NCI = Number of cases of isolation, OR = Occurrence remarks.

H= High occurrence; more than 12 samples out of 24 samples, M= Moderate occurrence; between 6-11 samples, L= Low occurrence; between 3-5 samples, R= Rare occurrence; less than 3 samples.

TABLE 4. Total counts (calculated per gm soil), percentage total counts (calculated per total fungi), number of cases of isolation (out of 24 samples) and occurrence remarks of hydrocarbon degrading fungi isolated from solar polluted soils collected from three governorates on 1% crude oil Czapek's agar medium at 28 ±1°C.

Governorate	El-Minya				Assiut				Sohag				Total			
Fungal species	TC	%TC	NCI	OR	TC	%TC	NCI	OR	TC	%TC	NCI	OR	TC	%TC	NCI	OR
Absidia cylindrospora	0	0	0	0	0	0	0	0	200	3.79	1	L	200	0.96	1	R
Aspergillus	8633.2	92.49	8	H	5966.4	97.81	8	H	4566.6	86.71	7	H	19166.2	92.59	23	H
Aspergillus awamori	0	0	0	0	400	6.56	2	L	400	7.59	2	L	800	3.86	4	L
Aspergillus flavus	1066.6	11.43	6	H	299.9	4.92	4	M	1200	22.78	3	M	2566.5	12.39	13	H
Aspergillus fumigatus	800	8.57	5	H	1266.6	20.76	5	H	566.6	10.76	3	M	2633.2	12.72	13	H
Aspergillus japonicus	66.6	0.71	1	L	0	0	0	0	0	0	0	0	66.6	0.32	1	R
Aspergillus niger	1500	16.07	4	M	66.6	1.09	2	L	1000	18.98	3	M	2566.6	12.39	9	M
Aspergillus terreus	5200	55.71	5	H	3933.3	64.48	6	H	1400	26.58	4	M	10533.3	50.88	15	H
Chrysosporium keratinophilum	0	0	0	0	0	0	0	0	200	3.79	2	L	200	0.96	2	R
Fusarium verticilloides	100	1.07	1	L	0	0	0	0	0	0	0	0	100	0.48	1	R
Mucor hiemalis	0	0	0	0	0	0	0	0	66.6	1.26	2	L	66.6	0.32	2	R
Penicillium	0	0	0	0	33.3	0.546	1	L	199.8	3.79	4	M	233.1	1.13	5	L
Penicillium chrysogenum	0	0	0	0	0	0	0	0	66.6	1.26	2	L	66.6	0.32	2	R
Penicillium glabrum	0	0	0	0	33.3	0.54	1	L	0	0	0	0	33.3	0.16	1	R
Penicillium oxalicum	0	0	0	0	0	0	0	0	33.3	0.63	1	L	33.3	0.16	1	R
Penicillium purpurogenum	0	0	0	0	0	0	0	0	99.9	1.89	2	L	99.9	0.48	2	R
Rhizopus	0	0	0	0	100	1.64	1	L	33.3	0.632	1	L	133.3	0.64	2	R
Rhizopus oryzae	0	0	0	0	0	0	0	0	33.3	0.63	1	L	33.3	0.16	1	R
Rhizopus stolonifer	0	0	0	0	100	1.64	1	L	0	0	0	0	100	0.48	1	R
Ulocladium chartarum	600	1.64	1	L	0	0	0	0	0	0	0	0	600	2.89	1	R
Total count	9333.2	100			6099.7	100			5266.3	100			20699.2	100		
No. of genera	3				3				6				8			
No. of species & varieties	7				7				12				17			

TC = Total counts, %TC = Percentage of total counts, NCI = Number of cases of isolation, OR = Occurrence remarks.

H= High occurrence; more than 12 samples out of 24 samples, M= Moderate occurrence; between 6-11 samples, L= Low occurrence; between 3-5 samples, R= Rare occurrence; less than 3 samples.

TABLE 5. Screening of 40 fungal isolates for crude oil utilization.

No.	Fungal isolates	Visual growth	Fungal dry mass (mg/30 ml culture)	Remarks
1	*Absidia cylindrospora*	+	68.33 ± 4.11	H
2	*Aspergillus awamorii* (1)	-	0	-
3	*Aspergillus awamorii* (2)	+	10.33 ± 2.62	L
4	*Aspergillus flavus* (1)	+	42.33 ± 8.18	M
5	*Aspergillus flavus* (2)	+	14 ± 7.12	L
6	*Aspergillus flavus* (3)	+	29.66 ± 6.94	L
7	*Aspergillus fumigatus* (1)	+	22 ± 0.82	L
8	*Aspergillus fumigatus* (2)	+	118 ± 2.94	H
9	*Aspergillus fumigatus* (3)	+	96 ± 6.68	H
10	*Aspergillus fumigatus* (4)	+	70.33 ± 3.86	H
11	*Aspergillus fumigatus* (6)	+	25 ± 2.45	L
12	*Aspergillus fumigatus* (7)	+	55 ± 6.68	M
13	*Aspergillus fumigatus* (8)	+	80.33 ± 2.05	H
14	*Aspergillus japonicus*	+	13.33 ± 3.30	L
15	*Aspergillus niger* (1)	+	18.66 ± 3.09	L
16	*Aspergillus niger* (2)	+	10.66 ± 1.70	L
17	*Aspergillus terreus (1)*	+	35 ± 4.32	M
18	*Aspergillus terreus (2)*	+	60 ± 5.10	H
19	*Aspergillus terreus (3)*	+	68.33 ± 3.30	H
20	*Aspergillus terreus (4)*	+	79 ± 6.53	H
21	*Aspergillus terreus (5)*	+	69.33 ± 6.60	H
22	*Aspergillus versicolor*	+	58 ± 3.74	M
23	*Chrysosporium keratinophilum* (1)	+	25 ± 7.26	L
24	*Chrysosporium keratinophilum* (2)	+	11 ± 2.45	L
25	*Chrysosporium tropicum*	-	0	-
26	*Cochliobolus specifer*	+	106 ± 5.72	H
27	*Fusarium oxysporum*	+	75 ± 4.32	H
28	*Fusarium solani*	+	66.66 ± 6.13	H
29	*Fusarium verticilloides*	+	80.66 ± 3.40	H
30	*Penicillium chrysogenum* (1)	+	10.33 ± 2.05	L
31	*Penicillium chrysogenum* (2)	+	35.66 ± 4.99	M
32	*Penicillium purpurogenum*	+	17 ± 4.90	L
33	*Rhizopus oryzae*	+	59 ± 4.90	M
34	*Rhizopus stolonifer*	-	0	-
35	*Syncephalastrum racemosum*	-	0	-
36	*Stachybotrys chartarum*	+	85.66 ± 8.06	H
37	*Trichoderma harzianum*	-	0	-
38	*Trichoderma harzianum*	-	0	-
39	*Ulocladium chartarum*	-	0	-
40	*Ulocladium chartarum*	-	0	-

H: Highly crude oil utilizers higher or equal to 60 mg dry mass, M: Moderate crude oil utilizers 30-59 mg dry mass, L: low crude oil utilizers less than 30 mg dry mass/30 ml culture.

TABLE 6. Screening of 40 fungal isolates for lipase production.

No.	Fungal isolates	Visual growth	Fungal mass (mg/30 ml culture)	Lipase activity (U/mL)	Yield (U/30 ml culture)	Extracellular protein (mg/ml)	Specific activity (U/mg protein)	Remarks
1	*Absidia cylindrospora*	+	103±4.32	8.91 ± 0.13	267.39±3.96	0.18 ±0.004	48.84±1.43	M
2	*Aspergillus awamorii* (1)	+	28±2.16	0	0	0	0	-
3	*Aspergillus awamorii* (2)	+	16±3.56	2.2 ± 0.31	66.05±9.14	0.1±0.008	22.01±4.55	L
4	*Aspergillus flavus* (1)	+	49±2.16	11.4 ± 0.24	342.12±7.29	0.39±0.004	29.12±0.43	H
5	*Aspergillus flavus* (2)	+	73±2.29	10.95 ± 0.01	328.63±0.56	0.32±0.009	34.5±0.97	H
6	*Aspergillus flavus* (3)	+	57±4.32	9.73 ± 0.77	291.96±23.2	0.19±0.006	50.99±4.32	M
7	*Aspergillus fumigatus* (1)	+	117±4.42	10.13 ± 0.26	304.07±7.95	0.32±0.004	31.67±0.67	H
8	*Aspergillus fumigatus* (2)	+	77±4.31	11.06 ± 0.03	331.74±1.01	0.34±0.007	32.6±0.66	H
9	*Aspergillus fumigatus* (3)	+	77±6.62	7.99 ± 0.3	239.72±9.06	0.18±0.01	44.59±4.39	M
10	*Aspergillus fumigatus* (4)	+	79±3.26	9.49 ± 0.05	284.69±1.72	0.28±0.005	33.39±0.62	M
11	*Aspergillus fumigatus* (5)	+	21±5.88	2.42 ± 0.4	72.62±12.03	0.16±0.005	14.82±2.8	L
12	*Aspergillus fumigatus* (6)	+	28±4.32	1.27 ± 0.01	38.02±0.43	0.11±0.006	11.43±0.76	L
13	*Aspergillus fumigatus* (7)	+	92±2.16	9.94 ± 0.25	298.18±7.45	0.24±0.003	41.99±0.56	M
14	*Aspergillus japonicus*	+	23±1.63	0.85 ± 0.04	25.57±1.42	0.16±0.005	5.22±0.14	L
15	*Aspergillus niger* (1)	+	24±3.55	0.79 ± 0.02	23.84±0.84	0.12±0.002	6.622±0.35	L
16	*Aspergillus niger* (2)	+	19±2.45	0.7 ± 0.02	21.07±0.75	0.11±0.006	6.689±0.27	L
17	*Aspergillus terreus (1)*	+	45±5.71	9.24±0.36	277.1±11.1	0.32±0.004	29.08±0.85	M
18	*Aspergillus terreus (2)*	+	44±4.54	11.58 ± 0.22	347.66±6.6	0.27±0.01	42.39±2.26	H
19	*Aspergillus terreus (3)*	+	85±1.66	11.51 ± 0.42	345.24±12.6	0.25±0.01	45.73±2.22	H
20	*Aspergillus terreus (4)*	+	30±2.94	11.14 ± 0.19	334.16±5.88	0.35±0.008	31.82±0.52	H
21	*Aspergillus terreus (5)*	+	62±2.92	10.83 ± 0.49	324.82±14.8	0.16±0.005	67.67±5.04	H
22	*Aspergillus versicolor*	+	0	0	0	0	0	0
23	*Chrysosporium keratinophilum* (1)	+	18±2.44	0.68 ± 0.03	20.38±0.85	0.16±0.008	4.27±0.29	L
24	*Chrysosporium keratinophilum* (2)	+	13±3.65	1.32 ± 0.05	39.75±1.7	0.1±0.007	13.25±1.09	L
25	*Chrysosporium tropicum*	+	19±2.45	0	0	0	0	-
26	*Cochliobolus specifer*	+	76±2.16	12.23 ± 0.28	367.03±8.65	0.37±0.01	33.29±0.77	H
27	*Fusarium oxysporum*	+	90±3.74	9.96 ± 0.27	298.87±8.19	0.28±0.007	34.75±0.19	M
28	*Fusarium solani*	+	107±1.63	9.98 ± 0.13	299.57±3.95	0.28±0.006	35.35±1.23	M
29	*Fusarium verticilloides*	+	75±2.45	12.28 ± 0.11	368.41±3.47	0.38±0.009	32.44±0.81	H
30	*Penicillium chrysogenum* (1)	+	42±3.29	0.86 ± 0.04	25.91±1.42	0.12±0.005	7.35±0.53	L
31	*Penicillium chrysogenum* (2)	+	22±2.45	10.11 ± 0.31	303.37±1.06	0.28±0.005	2.48±0.12	H
32	*Penicillium purpurogenum*	+	14±1.63	0.72 ± 0.03	21.76±1.02	0.31±0.007	9.22±0.62	L
33	*Rhizopus oryzae*	+	28±2.44	2.86 ± 0.15	85.76±4.53	0.21±0.006	13.56±1.11	L
34	*Rhizopus stolonifer*	-	0	0	0	0	0	-
35	*Stachybotrys chartarum*	+	35±3.26	1.09 ± 0.05	32.83±1.72	0.19±0.002	5.76±0.29	L
36	*Syncephalastrum racemosum*	-	0	0	0	0	0	-
37	*Trichoderma harzianum*	+	34±1.64	0	0	0	0	-
38	*Trichoderma harzianum*	-	0	0	0	0	0	-
39	*Ulocladium chartarum*	+	30±4.54	0	0	0	0	-
40	*Ulocladium chartarum*	-	0	0	0	0	0	-

H: Activity more than 10 U/ml, M: Activity 5-10 U/ml and l: Activity less than 5 U/ml.

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Okerentugba and Ezeronye (2003) isolated *Penicillium, Aspergillus* and *Rhizopus* from water sample polluted with fuel oil collected from New Calabar River located in the Niger Delta. Also, Elshafie *et al.* (2007) reported that they isolated *A. niger, A. ochraceus and P. chrysogenum* from tar balls collected from Oman beaches at Al-Qurum, AL-Hail and Al-Sawadi.

Obire *et al.* (2008) reported that they isolated *Aspergillus* for cow dung and poultry droppings and recorded their abilities as bioremediating agents from petroleum polluted soils at Rivers State in Nigeria. Okafor *et al.* (2009) isolated *Aspergillus versicolor* and *Aspergillus niger* from oil polluted soil samples in Nigeria and reported that they exhibited above 98% degradation efficiency for polycyclic aromatic hydrocarbon. Also, Al-Ghamdi (2011) reported that *Aspergillus flavus, A. niger, A. terreus, A. ochraceus,* and *Trichoderma sp.,* isolated from polluted soils collected from a mechanic workshops in Saudi Arabia, were able to utilize solar as sole carbon source.

The ability to isolate high numbers of certain oil degrading microorganisms from oil polluted environment is commonly taken as evidence that these microorganisms are active degraders in the environment. During our current study 40 fungal isolates belonging to 10 genera have been tested for their ability to grow and utilize crude oil. It has been found that out of the 40 isolates only 32 could grow and 8 fungal isolates couldn't. All differences between isolates in utilization of crude oil can be explained on the fact that the degradation capability of a compound is related to the enzymes production and enzymes activities of fungal isolate (Colombo *et al.,* 1996).

Lipase enzyme activity has been used as biochemical and biological parameter for hydrocarbon degradation and demonstrated as an excellent indicator for monitoring depollution of hydrocarbon polluted soils (Riffaldi *et al.,* 2006). In our current study, out of the 40 fungal isolates that have been grown on basal salt medium, only 35 were able to grow, of which 6 isolates grew without producing enzyme and 5 fungal isolates couldn't grow. *Fusarium verticilloides* isolated from solar polluted soil at El-Minya governorate showed the highest lipase activity (12.28 U/ml) and the highest yield (368 U/30ml culture). In agreement with our results it has been reported by Savitha and Ratledge (1991) that the genera *Aspergillus, Fusarium, Penicillium* and *Rhizopus* have been noted as lipase producers with desirable properties, which would have potential applications in a number of different areas.

Conclusion

The results presented in this study suggested that fungi isolated from crude oil polluted soils can be potentially used in lipase production as well as in bioremediation of polluted soils.

TABLE 4. Total counts (calculated per gm soil), percentage total counts (calculated per total fungi), number of cases of isolation (out of 24 samples) and occurrence remarks of hydrocarbon degrading fungi isolated from solar polluted soils collected from three governorates on 1% crude oil Czapek's agar medium at 28 ±1°C.

Governorate	El-Minya				Assiut				Sohag				Total			
Fungal species	TC	%TC	NCI	OR	TC	%TC	NCI	OR	TC	%TC	NCI	OR	TC	%TC	NCI	OR
Absidia cylindrospora	0	0	0	0	0	0	0	0	200	3.79	1	L	200	0.96	1	R
Aspergillus	8633.2	92.49	8	H	5966.4	97.81	8	H	4566.6	86.71	7	H	19166.2	92.59	23	H
Aspergillus awamorii	0	0	0	0	400	6.56	2	L	400	7.59	2	L	800	3.86	4	L
Aspergillus flavus	1066.6	11.43	6	H	299.9	4.92	4	M	1200	22.78	3	M	2566.5	12.39	13	H
Aspergillus fumigatus	800	8.57	5	H	1266.6	20.76	5	H	566.6	10.76	3	M	2633.2	12.72	13	H
Aspergillus japonicus	66.6	0.71	1	L	0	0	0	0	0	0	0	0	66.6	0.32	1	R
Aspergillus niger	1500	16.07	4	M	66.6	1.09	2	L	1000	18.98	3	M	2566.6	12.39	9	M
Aspergillus terreus	5200	55.71	5	H	3933.3	64.48	6	H	1400	26.58	4	M	10533.3	50.88	15	H
Chrysosporium keratinophilum	0	0	0	0	0	0	0	0	200	3.79	2	L	200	0.96	2	R
Fusarium verticilloides	100	1.07	1	L	0	0	0	0	0	0	0	0	100	0.48	1	R
Mucor hiemalis	0	0	0	0	0	0	0	0	66.6	1.26	2	L	66.6	0.32	2	R
Penicillium	0	0	0	0	33.3	0.546	1	L	199.8	3.79	4	M	233.1	1.13	5	L
Penicillium chrysogenum	0	0	0	0	0	0	0	0	66.6	1.26	2	L	66.6	0.32	2	R
Penicillium glabrum	0	0	0	0	33.3	0.54	1	L	0	0	0	0	33.3	0.16	1	R
Penicillium oxalicum	0	0	0	0	0	0	0	0	33.3	0.63	1	L	33.3	0.16	1	R
Penicillium purpurogenum	0	0	0	0	0	0	0	0	99.9	1.89	2	L	99.9	0.48	2	R
Rhizopus	0	0	0	0	100	1.64	1	L	33.3	0.632	1	L	133.3	0.64	2	R
Rhizopus oryzae	0	0	0	0	0	0	0	0	33.3	0.63	1	L	33.3	0.16	1	R
Rhizopus stolonifer	0	0	0	0	100	1.64	1	L	0	0	0	0	100	0.48	1	R
Ulocladium chartarum	600	1.64	1	L	0	0	0	0	0	0	0	0	600	2.89	1	R
Total count	9333.2	100			6099.7	100			5266.3	100			20699.2	100		
No. of genera	3				3				6				8			
No. of species & varieties	7				7				12				17			

TC = Total counts, %TC = Percentage of total counts, NCI = Number of cases of isolation, OR = Occurrence remarks.

H= High occurrence; more than 12 samples out of 24 samples, M= Moderate occurrence; between 6-11 samples, L= Low occurrence; between 3-5 samples, R= Rare occurrence; less than 3 samples.

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الميكوفلورا المعزولــة من التربه الملوثــة بالمــازوت والسولار في صعيد مصر

غادة عبد المنصف محمود ، مصطفي محمد منصور قطب ، فتحي محمد سيد مرسي و مجدي محمد خليل باجي
قسم النبات – كلية العلوم – جامعة أسيوط – مصر .

تم خلال هذه الدراسة عزل وتعريف 48 نوعا من الفطريات و صنفين تنتمي الى 12 جنسا من الفطريات على البيئتين الغذائيين الجلكوز- كزابك آجار و نفط خام – كزابك آجار من 48 عينة تربة ملوثة بالمازوت والسولار. تم تجميع التربة الملوثة بالهيدروكاربونات من ثلاث محافظات بصعيد مصر من صعيد مصر (المنيا – أسيوط – سوهاج). كان جنس الاسبرجيللس أكثر الاجناس الشائعة فى هذه الدراسة والذى تم عزله من الثلاث محافظات من التربة الملوثة بالمازوت والسولار وكان من أكثر الانواع شيوعا والذى تم عزله من التربة الملوثة بالمازوت هو اسبرجيللس فيومجاتس ، بينما كانت الانواع اسبرجيللس فلافس واسبرجيللس فيومجاتس واسبرجيللس تيريس اكثر الانواع شيوعا فى التربة الملوثة بالسولار. تم اختبار قدرة 40 عزلة فطرية تنتمي الى 22 جنس على استهلاك النفط الخام من البيئة الغذائيـة نفط خـام كزابك آجـار السائلة عند 28° من بين هذه العــزلات وجد ان 32 عزلة استطاعت النمو واستهلاك النفط الخام. أنزيم الليبيز يعتبر من الانزيمات التى تساهم فى استهلاك النفط الخام لذلك تم اختبار قدرة 40 عزلة على انتاج هذا الانزيم ووجد ان 35 عزلة لها القدرة على النمو وانتاج الانزيم. أظهرت النتائج قدرة فطرة الفيوزاريم فيرتيسيليود على انتاج كمية كبيرة من انزيم الليبيز منتجآ 12.28 وحدة/مللى.