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Full Length Research Paper

Fungal isolates and their toxicity from different ecosystems in Jeddah, Saudi Arabia

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The fact that toxic species do not always produce toxins and that other species not known to produce toxins were found to be toxic in some environments have been previously reported. However, different fungal species behave unexpectedly in different ecosystems. That is why the main objective of this work was to detect the toxicity of some fungal species existing in some environmentally important ecosystem in Jeddah in order to find a correlation between some of these environmental factors and the detected toxicity. The aim is to use some of the isolated non toxic strains that are capable of acting upon some environmental pollutant as a bioremediation approach. Forty nine fungal isolates were isolated from six different sources and ecosystems in both terrestrial and marine environment (agricultural soil, wheat grain, sewage dump, oily sewage dump, soil around car oil dump and marine fauna). Aflatoxins were detected in 18.4% of the total isolates. All the isolates from marine environment were non toxic to brine shrimp.

Key words: Mycoflora, ecosystems, mycotoxins, brine shrimp bioassay, bioremediation, Jeddah, Saudi Arabia.

INTRODUCTION

Some filamentous fungi are capable of producing low toxic molecular weight compounds referred to as mycotoxins, which contaminate food and feeds. Mycotoxins are unavoidably consumed or ingested by animals or humans. Severe health problems and death have occurred from mycotoxin consumption (Peraica et al., 1999; Creppy, 2002) and agricultural and natural ecosystems of plants are affected by climate change (Miraglia et al., 2009). Mycotoxins are climate, plant and method of storage dependent and are also influenced by non-infectious factors (e.g. bioavailability of micro-nutrients, insect damage and other pests attack) that are in turn driven by climatic conditions. Climate represents the key agro-ecosystem driving force of fungal colonization and mycotoxin production (Magan et al., 2003). Global warming and climate change certainly affect the occurrence of mycotoxins (Russell et al., 2009). Also, the production of mycotoxins is mainly affected by the fungi species and substrate type. Agricultural soil is an important substrate that researchers focus their attention on in order to explore different mycoflora and their toxicity as this is the first step to contaminating human food by mycotoxins. Mycological survey of agricultural soil reported the presence of six genera of filamentous fungi; *Aspergillus, Fusarium, Penicillium, Trichoderma, Cladosporium* and *Alternaria*. It is not only the type of agricultural soil but also some of the agricultural practices that affect the frequency of some fungal genera (Nesci et al., 2006).

A closely related substrate to the agricultural soil is grain. It is harvested, dried and then stored on farm or in silos for medium/long term storage. Interactions between spoilage, mycotoxigenic fungi and insect pests occur in stored grain ecosystems and this can further influence contamination with mycotoxins. The key mycotoxigenic moulds in partially dried grain are *Penicillium verrucosum* (Ochratoxin) in damp cool climates of Northern Europe and *Aspergillus flavus* (Aflatoxins), *Aspergillus ochraceus* (Ochratoxin) and some *Fusarium* species (Fumonisins and Trichothecenes) on temperate and tropical cereals. Studies on the ecology of these species have resulted in the modeling of germination, growth and mycotoxin minima and prediction of fungal contamination levels which may lead to mycotoxin contamination above the tolerable

Abbreviations: PDA, Potato dextrose agar; **YES,** yeast extract sucrose; **TLC,** thin layer chromatography.

legislative limits (Magan and Aldred, 2007). The sewage environment is another ecosystem that contains different mycoflora and mycotoxigenic profile. Fungi show capacity to absorb significant amount of metals that exist in some drainage system in their cell wall or by extracellular polysaccharide slime (Dasa et al., 2009). In cases where fungi are used as a tool for bioremediation, the first step of transferring the knowledge of the roles of fungi into technology development would require selection of species, followed by series of steps such as substrate design, enzymatic processes and operational parameters like temperature, flow rate, pressure, pH and residence time. As a result, different fungal isolates were used, long ago, for specific food, chemical and drug industries. Since then, new fungal isolates in special environment with high stress condition, that may produce novel fungal strains with unique characteristics which could be useful in biotechnology or bioremediation of environmental pollutants, were under wide research interest. During a project to explore the fungal diversity of microbial mats growing in the benthic environment of Antarctic Lakes, Brunati et al. (2009) reported that cold-tolerant cosmopolitan hyphomycetes such as *Penicillium, Aspergillus, Beauveria* and *Cladosporium* showed antimicrobial activity against gram-positive *Staphyloccus aureus*, gram-negative *Escherichia coli* and *Candida albicans*.

Another application is the use of these isolates, which have biodegradation potential, as one of the strategies for controlling environmental contamination using petroleum and its derivatives (Atlas, 1995b). Bioremediation agents have recently been applied to clean-up efforts after oil spills. These agents may contain inorganic or organic fertilizers, oil degradation microorganisms and/or seed cultures of oil-degrading microorganisms (Hoff, 1993; Atlas, 1995a; Swannell et al., 1996). Fungal isolates that are capable of living in oily condition would be a good candidate for such approach. Miranda et al. (2007) reported that cultures identified as *Rhodotorula aurantiaca* and *Candida ernobii* showed potential ability of these yeasts in degrading diesel oil. Also, Bento and Gaylarde (2001) reported that common fungal isolates from refineries and distribution systems, *Aspergillus niger* and *Aspergillus fumigates* as well as other fungal isolates, grew equally well in laboratory diesel/water systems with or without a chemical additive mixture. However, it is crucial to ensure the safety of any organism that is used in biotechnology even with those that will be used in open environment. That is why the toxicity of fungal isolates that may be used in biotechnology or biocontrol of environmental pollutant should be monitored. Therefore, the objective of the current study is to explore fungal communities that occur in some environmentally important ecosystem in Jeddah, to know whether such environment may trigger the appearance of toxic strains and to explore some important non toxic strains that are capable of acting upon some environmental pollutant as a bioremediation approach.

MATERIALS AND METHODS

Sampling sources

Forty nine fungal isolates were isolated from six different substrate sources and different ecosystems in both terrestrial and marine environment of Jeddah, Saudi Arabia. Agricultural soil, wheat grain, sewage dump, oily sewage dump, soil around car oil dump and marine fauna were the different sources of the collected samples (Table1).

Mycotoxins standards

Sigma standards (Sigma, chemical company, USA) of aflatoxins (B_1, B_2, G_1 and G_2) (AF) were used to prepare a stock standard solution according to the AOAC (2000) at concentration of 10 µg/ml using benzene: acetonitrile (98:2, v/v). Stock standard solutions of aflatoxins (AFB1, B2, G1, and G2) were prepared. The stock solution was diluted as appropriate, to obtain a working standard solution with a concentration of 1 µg/ml.

Culturing for fungal isolation and identification

Collected samples were cultured over Czapek (CZ), Potato Dextrose Agar (PDA) and malt extract agar media to isolate the fungal species that were present in these samples. The colors of these isolates on the different media and their morphological features under the light microscope were used to identify the different species (Raper and Fennell, 1965; Von Arx, 1974; Nelson et al., 1983; Mislivec et al., 1992; Nelson, 1992).

Preparation of spore suspensions

Purified fungal strains were regenerated on PDA slants at 28°C for one week to obtain sufficient inoculums. Slants were shaken with 5 ml of sterile Tween 80 (0.05%) and 2.0 ml of the resulting suspension of spores were used as inoculums.

Culturing for fungal toxicity

Yeast extract sucrose (YES) media as described by Tsubouchi et al. (1987) was used to grow isolated cultures in order to encourage them for toxin production. 100 ml portions of YES medium in Erlenmeyer flasks were inoculated by 1 ml of spore suspension with a concentration of 10^6 spores/ml and then incubated at 25 - 28°C for two weeks.

Mycotoxins extraction

The liquid YES cultures were filtered and the mycotoxins were extracted from both the cakes and the filtrates with chloroform. Mycotoxin chloroform extracts were then evaporated to dryness on steam bath. The residue was quantitatively transferred to a small vial with chloroform, evaporated to dryness on steam bath under nitrogen and reserved for brine shrimp bioassay and thin layer chromatography (TLC) methods.

Detection of mycotoxicity by brine shrimp bioassay

Brine shrimp (*Artemia salina* Leach) bioassay (Kiviranta et al., 1991) was used to detect any toxic activity in the fungal isolates extracts. One day old shrimps were placed in vials containing 5 ml

Table 1. The list of the fungal species isolated from the different sources in Jeddah.

Sample number	Sample source	Identification
1	Agricultural soil	*P. corylophilum*
2	Agricultural soil	*A. japonicas*
3	Agricultural soil	*P. corylophilum*
4	Agricultural soil	*A. versicolor*
5	Agricultural soil	*A. versicolor*
6	Marine fauna	*A. japonicas*
7	Marine fauna	*A. parasiticus*
8	Marine fauna	*P. rubrum*
9	Marine fauna	*A. niger*
10	Marine fauna	*A. versicolor*
11	Sewage dump	*P. citrinum*
12	Sewage dump	*P. corylophilum*
13	Sewage dump	*A. parasiticus*
14	Sewage dump	*A. terreus*
15	Sewage dump	*Trichoderma* sp.
16	Sewage dump	*A. niger*
17	Sewage dump	*A. flavus*
18	Sewage dump	*A. parasiticus*
19	Sewage dump	*P. citrinum*
20	Oily sewage dump	*Penicillium* sp.
21	Oily sewage dump	*A. niger*
22	Oily sewage dump	*A. flavus*
23	Soil around car oil dump	*E. nidulans*
24	Soil around car oil dump	*A. tamari*
25	Soil around car oil dump	*A. niger*
26	Soil around car oil dump	*A. tamari*
27	Soil around car oil dump	*A. terreus*
28	Soil around car oil dump	*E. nidulans*
29	Soil around car oil dump	*E. nidulans*
30	Soil around car oil dump	*A. niger*
31	Wheat grain	*A. versicolor*
32	Wheat grain	*A. alternate*
33	Wheat grain	*A. parasiticus*
34	Wheat grain	*P. canesens*
35	Wheat grain	*A. carneus*
36	Wheat grain	*A. parasiticus*
37	Wheat grain	*A. parasiticus*
38	Wheat grain	*A. flavus*
39	Wheat grain	*A. flavus*
40	Wheat grain	*Acremonium* sp.
41	Wheat grain	*A. flavus*
42	Wheat grain	*E. chievalieri*
43	Wheat grain	*A. niger*
44	Wheat grain	*A. tamari*
45	Wheat grain	*A. alternate*
46	Wheat grain	*A. flavus*
47	Wheat grain	*E. nidulans*
48	Wheat grain	*A. flavus*
49	Wheat grain	*A. carneus*

Table 2. Genera and species frequency within sample sources in Jeddah.

Genera (sample)	Species	No. of species within source	Sample frequency
Aspergillus	*A. flavus*	7	32
	A. parasiticus	6	
	A. niger	6	
	A. versicolor	4	
	A. tamari	3	
	A. japonicas	2	
	A. terreus	2	
	A. carneus	2	
Penicillium	*P. corylophilum*	3	8
	P. citrinum	2	
	P. rubrum	1	
	P. canesens	1	
	Penicillium sp	1	
Emericella	*E. nidulans*	4	4
Alternaria	*A. alternate*	2	2
Trichoderma	*Trichoderma sp*	1	1
Acremonium	*Acremonium* sp	1	1
Eurotium	*E. chievalieri*	1	1
Total	18	49	49

seawater (10 shrimps/vial). The dried extracts were dissolved in seawater to give concentration of 1500 ppm in each vial containing the 10 shrimps. The number of dead shrimps after 24 and 48 h was counted and percent mortality calculated.

Aflatoxins and ochratoxin determination by TLC

Merck ready-made TLC plates (Merck, Darmstadt, Germany) of 0.2 mm thickness of silica gel G 60 (without fluorescent indicator) supported by 20 × 20 cm^2 aluminum sheets were used for aflatoxins determinations. Tested and standard spots were placed at 1 cm intervals of 4 cm from bottom edge on an imaginary line (base line). Samples residues were redissolved in 100 µl chloroform then 10 µl of each sample and aflatoxins standard solution were spotted on the same plate. The plate was developed in a developing system (toluene: ethyl acetate: formic acid, 6:3:1 v/v/v) in saturated tank. The plates were examined under long wave ultraviolet light (UV, 365 nm); AFB_1 and B_2 had blue fluoresce, and G_1 and G_2 had green fluoresce. Fluorescent spots resembling aflatoxins and those having the same R_f were confirmed by spraying the TLC plates with 20% sulfuric acid; AFB_1 and B_2 had yellow fluoresce, while G_1 and G_2 had yellow- blue (AOAC, 2000).

RESULTS AND DISCUSSION

The fact that some toxigenic fungal species are not toxic or at least show low toxic profile in some substrates or within some environmental factors always raise the question of why they produce toxins and at what environmental conditions they do that. That is why the objective of this study is to see if the same fungal species that occur in different ecosystem toxicologically respond differently. A second objective was to explore non toxic strains that may be used as a safe biocontrol on some environmental pollutant. Out of the six different sources (agriculture soil, wheat grains, sewage dump, oily sewage dump, soil around car oil dump and marine fauna) that were explored in this study, 49 fungal isolates were identified as shown in Table 1. Also, *Aspergillus* and *Penicillium* consist of all the mycoflora of oily sewage dump, most of the sewage dump and soil around car oil dump. Davies and Westlake (1979) reported that 28 out of 34 fungi studied were capable of growing on a variety of crude oils. Kacprzak et al. (2005) reported that the most frequently occurring fungi in wastewater and sewage sludge were the genus *Penicillium* and they usually occupy about 50% of all studied communities. Also, *Aspergillus* sp. and *Penicillium* sp. were found to be the most frequent fungi among other fungal isolates in wastewater and sewage sludge (Kacprzak et al., 2003). The occurrence of mould fungi (*Aspergillus, Fusarium, Penicillium* or *Mucor*) in wastewater and sewage sludge, are probably connected with large amount of easily fermenting sugars in wastewater and sewage sludge (Kacprzak et al., 2005).

The highest frequency of the fungal isolates belonged to the genus *Aspergillus* with a frequency percentage of 65.3% followed by the genus *Penicilium* which constitute 16.32% of the total isolates. Snellman et al. (1988) showed that the species of *Aspergillus* and *Penicillium* were made up of 33 and 39% of fungi on tar balls, respectively (Table 2). The most frequent species within the studied samples were the probable aflatoxins producers; A. *flavus* and *Aspergillus parasiticus* with a frequency percentage of 14.3 and 12.2%, respectively.

This means that these two species constitute 26.5% of the total isolates; however, most of their occurrences (16.3% of the total isolates) were found in the wheat grain samples (Table 2). These results were confirmed by Boyd and Cotty (2001) who reported that much of the organic matter in soils is colonized by *A. flavus* and related fungi in warm semi-arid regions. On the contrary, Russell et al. (2009) suggested that tropical countries may become too inhospitable for conventional fungal growth and mycotoxin production, which may lead to the extinction of thermotolerant *A. flavus*.

The second most frequent species was *A. niger* with 12.2%, while *A. japonicas* constitute 4.1%. The increasing frequency of black *Aspergilli* was confirmed by other researchers who reported that high number of black *Aspergilli* was detected during harvest in four European regions in 2002 and 2003. The same trend was found in sampling carried out in 2001. This suggests that late ripening marks a profound change in the ecological factors affecting fungal conidiation, dissemination of conidia and fungal growth. In 2003, more black *Aspergilli* were isolated than in the two previous years. Perhaps, this was because 2003 was an extremely hot year in Spain. High temperatures could also explain the higher number of black *Aspergilli* found in Costers del Segre in 2002 and 2003 (Bellí et al., 2005).

A. flavus and *A. parasiticus* were neither found in the agricultural soil samples collected in this study nor in the soil around car oil dump. However, most of these two species were found in the wheat grain samples. This means that wheat samples were contaminated with these two species during storage for local samples or via imported wheat samples. *A. niger* was found in all the collected samples except in the agricultural soil samples. This indicates the wide spreading character of this species. The toxicity of the isolated fungal species was tested using brine shrimp bioassay. Three doses of the fungal extracts (500, 1500 and 3000 ppm) were used to detect the fungal isolates toxicity. The use of the high doses (1500 and 3000 ppm) was mainly to detect any traces of minor toxicity that may present in the collected isolates. Since the second objective of this study was to explore non-toxic species that could be used in biocontrol treatment of some pollutants, the use of such high doses of fungal extracts (1500 and 3000 ppm) will ensure the safety of these isolates. Table 3 show the mortality percentage of the brine shrimp after been subjected to the fungal extracts for 24 and 48 h. The toxic potency of the isolates as well as the safe non toxic species is clearly indicated in Table 3. All the isolates from the sewage dump environment proved to be toxic to brine shrimp in different percentage ranging from 60 -100%. However, most of the mortality percentage was 100%.

Out of the studied samples within the wheat grains source (at 3000 ppm), it was found that 16 out of 19 isolates (84.2%) were toxic to brine shrimp. In the case of agricultural environment, most of the agricultural soil isolates (80%) contained toxic species. Isolates that occur in oily environment showed lower toxicity where the toxicity ranged between 33.3% for oil sewage dump to 50% in the soil around car oil dump.

The toxicity to brine shrimp can detect the presence of a toxic molecule that may exist in the fungal isolates. However, this assay cannot detect which type of mycotoxins is produced by a certain fungal species. Therefore, levels of aflatoxins were detected by using the semi quantitative TLC method. The sample source and the fungal species along with aflatoxin concentration are recorded in Table 4. From Table 3, 34 fungal species of the studied samples were found to be toxic to brine shrimp (100% toxic at 3000 ppm). 9 isolates (26.5%) out of 34 toxic fungal species were capable of producing aflatoxins (Table 4). Out of these 9 species, five species were isolated from wheat grain source although Walsh (2009) predicted that due to the effect of global warming, mycotoxigenic moulds could become extinct. The result indicated in this study denied such assumption as 69.4% of the collected isolates were toxic. This means that global warming is most likely to increase the occur-rence of toxic fungi. Other reports confirmed such results where increased quantities were reported in the myco-toxigenic fungi of the tropical, warm, arid and semi-arid regions. A good example of these mycotoxigenic fungi are the aflatoxin producing fungi of which changes in climate result in large alterations in the quantity of these fungi (Bock et al., 2004; Shearer et al., 1992).

All isolates collected from the marine fauna showed no toxicity to brine shrimp bioassay at 500 and 1500 ppm after 24 h (Table 3). Although toxic species like *A. parasiticus* (isolate number 7) exist, it seems that this environment does not encourage mycotoxin production. However, this particular strain showed 60% mortality in brine shrimp after 48 h at 1500 ppm. This means that minor toxicity exists even after the culturing enrichment on YES medium. Also, the lowest level of recorded aflatoxins (8 ppb) was detected in this strain (Table 4). This means that the brine shrimp assay could not detect such low level of toxicity by aflatoxins till the 48 h of 1500 ppm. The non toxicity of the fungal isolate from marine environment was confirmed by Brunati et al. (2009), who found no toxicity of the fungal isolates of the Antarctic Lakes during his attempt to discover novel antibiotics from such environment. Although Elshafie et al. (2007) used 10 fungal isolates for potential use in oil pollution removal, no attempt was made for those isolates to check their toxicity. On the contrary, *A. parasiticus* showed moderate toxicity at 500 ppm (20 - 60%) and maximum toxicity at 1500 ppm (100%) after 24 h. Numbers 13 and 18 of the isolates were originally collected from the sewage dump (Table 3). However, low level (2 ppb of isolate 13) or no aflatoxins of isolate 18 were detected (Table 4). This means that the toxicity detected by brine shrimp was due to other mycotoxins rather than aflatoxins. The same trend was observed in isolate number 33 (*A.*

Table 3. Mycotoxicity detection of the isolated fungal species by brine shrimp bioassay.

Sample number	Identification	Sample source	500 ppm		1500 ppm		3000 ppm	
			Mortality % after 24 and 48 h					
			24	48	24	48	24	48
Control			0.0	0.0	0.0	3.3	0.0	0.0
1	*P. corylophilum*	Agricultural soil	0.0	20	0.0	60	30	70
2	*A. japonicus*	Agricultural soil	40	50	100	100	100	100
3	*P. corylophilum*	Agricultural soil	10	30	40	100	40	100
4	*A. versicolor*	Agricultural soil	20	40	90	100	90	100
5	*A. versicolor*	Agricultural soil	10	30	70	100	70	100
6	*A. japonicus*	Marine fauna	0.0	30	0.0	50	10	60
7	*A. parasiticus*	Marine fauna	0.0	0.0	0.0	40	20	80
8	*P. rubrum*	Marine fauna	0.0	20	0.0	40	0.0	40
9	*A. niger*	Marine fauna	0.0	20	0.0	30	10	40
10	*A. versicolor*	Marine fauna	0.0	10	0.0	40	20	70
11	*P. citrinum*	Sewage dump	30	60	100	100	100	100
12	*P. corylophilum*	Sewage dump	20	50	100	100	100	100
13	*A. parasiticus*	Sewage dump	30	50	100	100	100	100
14	*A. terreus*	Sewage dump	20	40	70	100	70	100
15	*Trichoderma* sp.	Sewage dump	30	40	100	100	100	100
16	*A. niger*	Sewage dump	30	50	100	100	100	100
17	*A. flavus*	Sewage dump	20	40	60	100	80	100
18	*A. parasiticus*	Sewage dump	20	60	100	100	100	100
19	*P. citrinum*	Sewage dump	30	50	100	100	100	100
20	*Penicillium* sp.	Oily sewage dump	30	60	100	100	100	100
21	*Aspergillus niger*	Oily sewage dump	0.0	0.0	0.0	20	30	50
22	*Aspergillus flavus*	Oily sewage dump	10	30	30	60	50	80
23	*E. nidulans*	Soil around car oil dump	80	100	100	100	100	100
24	*A. tamari*	Soil around car oil dump	20	50	90	100	100	100
25	*A. niger*	Soil around car oil dump	0.0	0.0	0.0	50	40	80
26	*A. tamari*	Soil around car oil dump	30	60	100	100	100	100
27	*A. terreus*	Soil around car oil dump	0.0	0.0	0.0	10	50	90
28	*E. nidulans*	Soil around car oil dump	0.0	20	0.0	60	30	80
29	*E. nidulans*	Soil around car oil dump	30	60	100	100	100	100
30	*A. niger*	Soil around car oil dump	0.0	0.0	0.0	10	30	40
31	*A. versicolor*	Wheat grain	30	50	70	100	100	100
32	*A. alternata*	Wheat grain	20	50	70	90	70	100
33	*A. parasiticus*	Wheat grain	30	60	100	100	100	100
34	*P. canesens*	Wheat grain	40	60	100	100	100	100
35	*A. carneus*	Wheat grain	30	50	80	100	100	100
36	*A. parasiticus*	Wheat grain	50	80	100	100	100	100
37	*A. parasiticus*	Wheat grain	60	80	100	100	100	100
38	*A. flavus*	Wheat grain	0.0	0.0	0.0	0.0	0.0	0.0
39	*A. flavus*	Wheat grain	10	30	50	70	70	100
40	*Acremonium* sp.	Wheat grain	20	40	100	100	100	100
41	*A. flavus*	Wheat grain	30	60	100	100	100	100
42	*E. chievalieri*	Wheat grain	10	60	80	100	100	100
43	*A. niger*	Wheat grain	20	40	80	90	100	100
44	*A. tamari*	Wheat grain	20	50	100	100	100	100
45	*A. alternata*	Wheat grain	30	60	100	100	100	100
46	*A. flavus*	Wheat grain	40	60	100	100	100	100
47	*E. nidulans*	Wheat grain	0.0	20	0.0	60	40	80
48	*A. flavus*	Wheat grain	30	60	70	100	100	100
49	*A. carneus*	Wheat grain	0.0	20	0.0	40	20	50

Table 4. Aflatoxin level by TLC of the toxic isolates and their source of sampling.

Isolate number	Sample source	Identification	Aflatoxin (ppb)				Total
			B_1	G_1	B_2	G_2	
5	Agricultural soil	*A. versicolor*				4	4
7	Marine fauna	*A. parasiticus*	6		2		8
13	Sewage dump	*A. parasiticus*		2			2
20	Oily sewage dump	*Penicillium* sp.	2	4	2	2	10
36	Wheat grain	*A. parasiticus*	8	10	8	10	36
37	Wheat grain	*A. parasiticus*	8	10	8	10	36
39	Wheat grain	*A. flavus*	10	6			16
43	Wheat grain	*A. niger*	8	8	10	4	30
48	Wheat grain	*A. flavus*	6	10			16

parasiticus in wheat grain). Similarly, *A. flavus* (isolate number 17, 22 and 41) showed toxicity by brine shrimp assay in different potency where no aflatoxins were detected in these isolates. This means that toxicity is due to other mycotoxins. It should be noted that these strains of *A. flavus* were isolated from different sources (sewage dump, oily sewage dump and wheat grain, respectively). However, they share the same characteristic of having toxicity and no aflatoxins production. It should be noted also that *A. parasiticus* and *A. flavus* of isolates number 13, 18, 17 and 22 that were originally collected from the sewage dump showed the same trend. They showed toxicity to brine shrimp without the presence of significant concentration of aflatoxins. Also, among all the isolates that produce aflatoxins, none of them was capable of producing both of these toxins together (Table 4). The effect of the substrate was clearly shown by the effect of wheat grain matrix on the toxin production of all *A. flavus* and *A. parasiticus* isolates except for one isolate (isolate number 38) as well as the effect of sewage dump in the toxicity of isolates number 13, 17, 18 and 22 (Table 3). Although *A. parasiticus* is a probable toxigenic specie, in marine ecosystem this species (isolate number 7) is non toxic as well as all isolates of this particular ecosystem. The same trend was observed for the toxic occurrence of *A. versicolor* in agricultural soil (isolates number 4 and 5) and in wheat grain (isolate number 31) where it was non toxic in the marine ecosystem (isolate number 10). It is not only the type of soil that affect the frequency of toxigenic fungi but also some of the agricultural practices as indicated by Nesci et al. (2006) who reported that the isolation frequency of *Aspergillus* increased in the no tillage and grazing practices treatments.

Although agricultural soil encourage the toxicity of *Aspergillus japonicas* (Table 3) where marine ecosystem suppress such toxicity, the same agricultural soil environment did not trigger the toxicity of *Penicillium corylophilum* (isolate number 1) where the same species is toxic in sewage dump ecosystem (isolate number 12). The second objective of the current study is to explore the presence of non toxic strains that can be used in the removal of environmental pollutants in different ecosystems. Compared with isolates of other fungi, *Aspergillus* and *Penicillium* isolates were reported to be rich in hydrocarbon assimilatory strains and were capable of crude oil degradation (Fedorak et al., 1984; Hashem, 1995). The results illustrated in Tables 3 and 4 show that fungi isolates from marine ecosystem in general could be considered as safe (Figure 1and 2). Moreover, *A. niger* in marine ecosystem (isolate number 9) showed the lowest toxicity after 48 h for maximum concentration of 1500 and 3000 ppm in brine shrimp bioassay. This result made *A. niger* a good candidate for biocontrol approach. The safety of *A. niger* was confirmed in other ecosystem where the isolate of *A. niger* in both the oily sewage dump (isolate number 21) and the soil around car oil dump (isolate number 25) showed no toxicity after 24 and 48 h with 20 and 50% mortality of 1500 and 3000 ppm respectively. Also *Penicillium rubrum* in marine ecosystem (isolate number 8) and *Penicillium corylophilum in* agricultural soil (isolate number 1) could be a second candidate for bioremediation. The fact that these strains (isolates number 21 and 25) are non toxic even at high concentrations of their extracts and were isolated from oily ecosystem could direct their use in the biodegradation of oil spills that may occur in some marine ecosystem. Bento and Gaylarde (2001) proved the ability of *A. niger* isolated from refineries and distribution systems to grow well in laboratory diesel/water systems. Also Elshafie et al. (2007) reported the abilities of *A. niger, A. ochraceus* and *P. chrysogenum* isolated from tar balls collected from the beaches of Oman to grow and degrade *n*-alkanes (C13 - C18) and crude oil.

In conclusion, good knowledge of fungal communities' structure has two important points. The first is the knowledge of long-term survival of human or animal toxigenic and pathogenic fungi (Kacprzak et al., 2005). Therefore, interactions with different ecosystems should be through well defined scientific data to ensure the safety of such interactions. The second is the potential use of some fungal species in bioremediation approaches of oil spills, wastewater from oil manufacturing plant (Chigusa et al.,

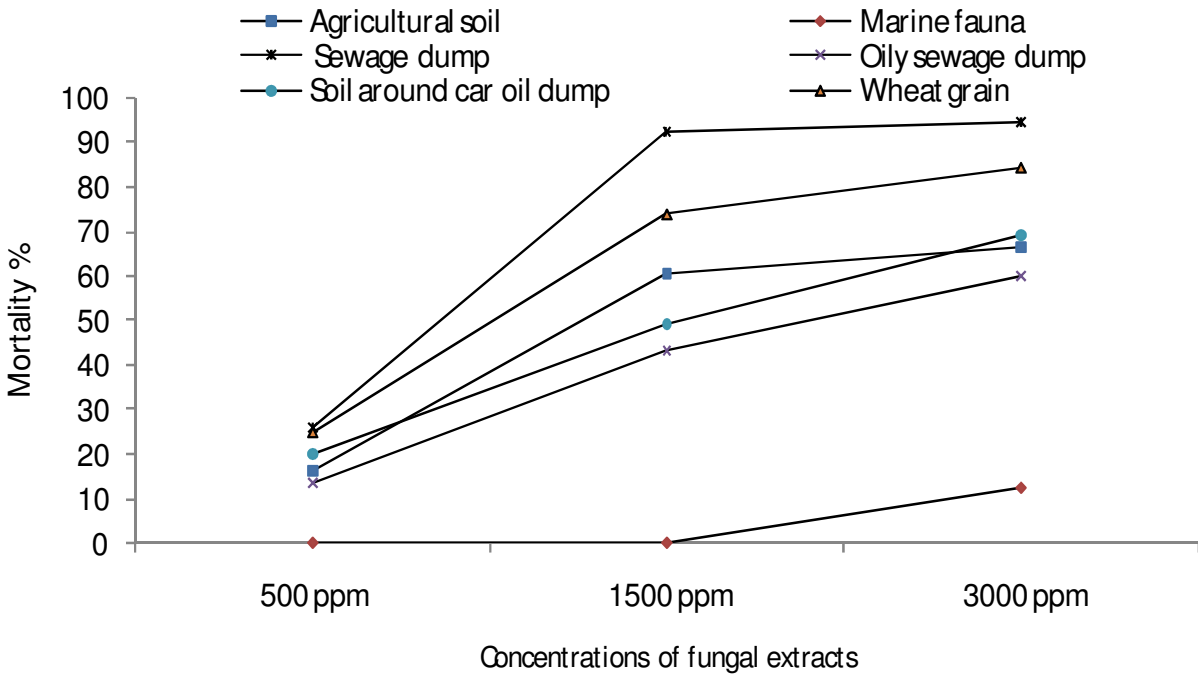

Figure 1. Effects of substrates on fungal toxicity using brine shrimp bioassay after 24 h.

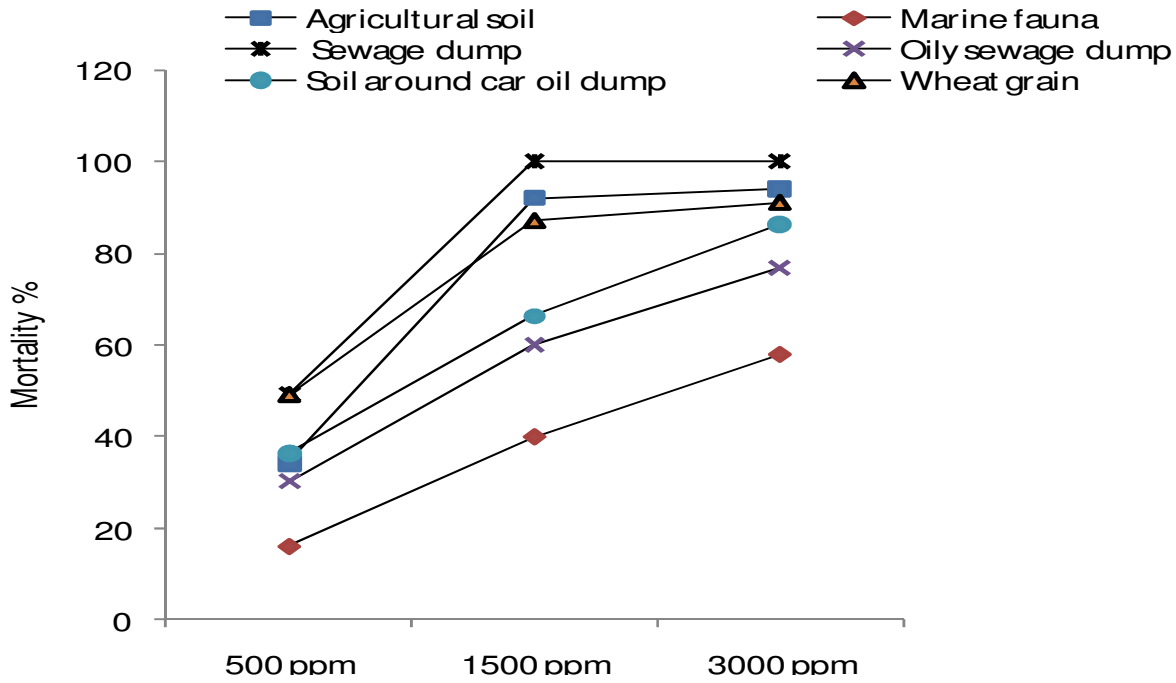

Figure 2. Effect of substrates on fungal toxicity using brine shrimp bioassay after 48 h concentrations of fungal extracts.

1996), municipal and industrial wastewater (Assadi and Jahangiri, 2001) as well as other environmental pollutant. It is of crucial importance to ensure the safety of the fungal isolates selected for such approach.

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