

Characterization of fungi transferred by dust storms in Kuwait and their plant pathogenicity

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Abstract Dust storms carry large amounts of plant detritus and microorganisms that may cause diseases in humans, animals or plants. These storms are frequent in Kuwait throughout the year. This research was conducted to identify the fungal species carried by the dust storms in Kuwait, originating from the northwesterly direction, with emphasis on plant pathogens. Fungi were isolated from settled dust samples and identified using established microbiological and molecular approaches. Fungal isolates identified as *Fusarium oxysporum* from settled dust were examined for pathogenicity using a number of crop plants. In total, 17 genera of fungi were identified in the dust samples. These fungi included plant pathogens or facultative plant parasites that were transported in the dust storms as viable propagules. The most common dust-carried fungi belonged to the genera *Fusarium*, *Alternaria*, *Ulocladium*, *Phoma*, *Aspergillus*, *Acremonium* and *Penicillium*. The *F. oxysporum* isolates that had been characterized by partial 18S rRNA gene sequencing were pathogenic, causing root and stem rot in tomato, bean and cucumber, but not squash.

Keywords Dust storms · Fungi · Plant pathogens · *Fusarium oxysporum*

1 Introduction

Dust and dust storms are natural phenomena in many parts of the world, especially in arid and semiarid regions. An estimated amount of 2.2 billion metric tons of soil and dried sediments is transported through the earth's atmosphere each year, mainly by dust storms (Griffin et al. 2001). Dust storms are caused by extreme daytime temperatures and/or high surface winds. In desert regions, dust is an important climate variable and can be transported for hundreds of kilometers to other regions (Prospero 1981; Reiff and Forbes 1986). The prevailing wind directions affecting Kuwait are the northwesterly winds, locally known as “Shamal” (Ali 1994). The Shamal winds are usually associated with dust (Daoud 1985).

Research interest in the development and occurrence of dust storms is based on public health concerns, the potential hazards associated with the dust load and the different components that constitute dust clouds including airborne pathogens, pesticides and chemical contaminants, which may directly affect humans and other organisms. Current concerns include pathogen propagules or inocula in the dust that can infect plants and animals. Fungi have an advantage over many other microorganisms in their production of spores that enhance their survival during transport in dust storms. Dust itself filters UV light and therefore protects the spores and enhances their survival within the dust cloud (Dufrene 2000). Fungal

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populations in dusts are quite diverse, and the extent of diversity is probably much greater than assumed thus far (Griffin et al. 2003; Kellogg et al. 2004; Griffin 2007). Some plant diseases are associated with dust storms (Bowden et al. 1971; Buchwaldt et al. 1996; Elias et al. 1991). The transport of pathogens by dust storms is an emerging area of research and includes, for example, the periodic transport of tobacco blue mold (*Peronospora tabacina* Adam) from Cuba to the southeastern USA (Davis and Monahan 1991), and of sugarcane rust caused by *Puccinia melanocephala* H. Syd. & P. Syd., to the Dominican Republic (Purdy et al. 1985).

The objective of this research was to isolate, characterize and compare fungi from dust samples collected during dust storms at various locations in Kuwait and to test their potential as plant pathogens.

2 Materials and methods

2.1 Sampling

Dust samples were collected during the period from 2007 to 2011 from the urban and farm areas in Kuwait

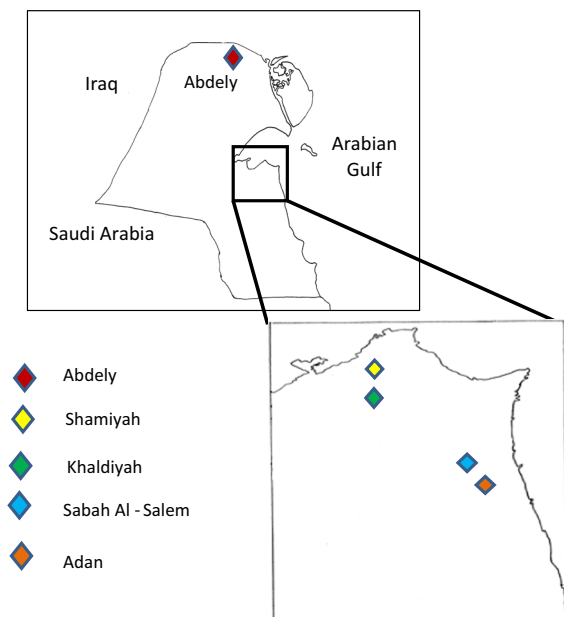


Fig. 1 Map of the State of Kuwait showing different areas from which dust samples were collected. These areas are Abdely, a farming area in the north and residential areas (shown on an inset map) of Shamiyah, Khaldiya, Sabah Al-Salem and Adan

(Fig. 1). Nine samples were collected during different dust storm events from Khaldiya, Shamiyah, Sabah Al-Salem, Adan and from the agricultural area of Abdely (Table 1). Surface-sterilized plastic sheets, 1 m in diameter, were placed on building rooftops (15 m high) for 3 h and left to receive the deposited dust. The samples were collected and labeled with relevant information and stored aseptically in cool dry conditions.

2.2 Enumeration and isolation of fungi

Under aseptic conditions, 0.27 g of each dust sample was transferred into a 100-ml sterile conical flask containing 35 ml of either sterile distilled water or a mineral medium (BG-11) (Stanier et al. 1971). Flasks were prepared in triplicate and coated with foil to inhibit algal growth and lower any possibility of contamination with other environmental fungi. Flasks were kept on a rotary shaker, 100 rpm, at room temperature for 1 day to break spore dormancy. After 1 week of incubation, a 1:10 (v/v) dilution was conducted under aseptic conditions. From the dilution, 0.2 ml was spread evenly on potato dextrose agar (PDA) medium amended with 0.05 g/ml streptomycin sulfate (Sigma-Aldrich, USA). Triplicate plates were prepared and incubated at room temperature for 1 week. The fungal colony-forming units (CFU) were counted, and the total numbers in one gram of dust were calculated.

Fungal colonies were subcultured by transferring hyphal tips from each colony onto a fresh PDA plate. When feasible, single-spore cultures were also

Table 1 A list of different dust samples used in this study, showing the code name, location and date of collection of each sample

No.	Sample	Location	Date
1	190507Kh	Khaldiya	19-5-2007
2	210208Kh	Khaldiya	21-2-2008
3	090208Kh	Khaldiya	9-2-2008
4	070610Kh	Khaldiya	7-6-2010
5	250311Kh	Khaldiya	25-3-2011
6	010710Ad	Adan	1-7-2010
7	300610Sh	Shamiyah	30-6-2010
8	030710Ss	Sabah Al-Salem	3-7-2010
9	070810Abd	Abdely	7-8-2010

prepared. Culture plates were sealed with parafilm and stored at room temperature. Isolates were subcultured every 2–3 months.

Fungi were isolated from infected plants by cutting the infected tissues into small pieces (2–5 mm squares) and surface sterilized by soaking the pieces in hydrogen peroxide (50 %) for 30–60 s. The sterilized pieces are aseptically transferred to Petri dishes containing standard potato dextrose agar (PDA) supplemented with streptomycin sulfate, at the rate of three to five pieces of tissues per Petri dish and incubated at room temperatures.

2.3 Identification of fungal isolates

Fungal cultures were sorted according to their morphological and cultural characteristics. Representative colonies of each group were used for phylogenetic characterization. The fungal hyphae and spores were stained with lacto-phenol or cotton blue for microscopic examination. The Riddell's classic slide culture method (Riddell 1950) was used to obtain spores for examination and photography using a light microscope (Olympus Vanox AHB S3, Japan).

2.4 DNA extraction

For extraction of DNA from fungal isolates, a piece of the fungal mycelium was suspended in 200 µl of TE buffer (Tris–EDTA, pH 8.0). The fungal mycelia were lysed by subjecting them to four freeze–thaw cycles, and the DNA was extracted using a PrepMan Ultra kit (Applied Biosystems, USA) following the manufacturer's instructions. The extracted DNA was examined for purity by electrophoresis (see below).

2.5 Amplification of the partial 18S rRNA genes

Partial 18S rRNA genes were amplified using a forward primer FF2 (5'-GGTTCTATTTTGTGGTTTCTA-3') and a reverse primer FR1 (5'-CTCTCAA TCTGTCAATCCTTATT-3'; Zhou et al. 2000). The purity of the 425 bp amplicons was checked by gel electrophoresis. For DNA amplification, an initial denaturation of DNA at 95 °C for 3 min was followed by 35 cycles of denaturation at 94 °C for 1 min, annealing at 54 °C for 1 min, and extension at 72 °C for 2 min. The samples were subjected to an additional extension step at 72 °C for 10 min at the end of the

amplification cycles. The purity of the 425 bp amplified products was examined by gel electrophoresis (0.5 wt%/vol agarose) and comparison with 100 bp ladder DNA size marker (Promega, USA).

2.6 DNA sequencing and analysis

Polymerase chain reaction (PCR) products were purified using the QIAquick PCR purification kit (QIAGEN, UK), following the manufacturer's instructions. Purified PCR products were sequenced using an ABI prism 3130/3130XL Genetic Analyzer (Applied Biosystems, USA). Sequences were aligned using BioEdit software (Hall 1999) and submitted for comparative analysis to the National Centre for Biotechnology Information (NCBI) online using the Basic Local Alignment Search Tool (BLAST) program (Wheeler et al. 2006). The sequences were compared with their closest match in the GenBank.

2.7 Pathogenicity of *Fusarium oxysporum* isolates

The test materials were seedlings of tomato (*Lycopersicon esculentum*), cucumber (*Cucumis sativus*), squash (*Cucurbita maxima*) and common bean (*Phaseolus vulgaris*). Seeds were surface sterilized with 1 % sodium hypochlorite (NaClO). The pathogenicity test was conducted in the laboratory, in a growth chamber and in a greenhouse. In the laboratory seedling bioassay, sterilized seeds were germinated in Petri dishes lined with moist, sterile filter papers until the seedlings were 9–10 cm tall, followed by inoculation with *F. oxysporum*. For the growth chamber and greenhouse bioassays, sterilized seeds were germinated until the 3–4 leaf stage in sterilized local soil (sandy loam) dispensed in 1.5-l plastic pots. Four *F. oxysporum* strains from various dust samples were tested using either 10⁶ aliquots of conidia (hemocytometric count) or 5-mm-diameter plugs of actively growing mycelia. Twelve replicates were used for each treatment. Each experiment was carried out twice.

For the laboratory seedling bioassay, actively growing mycelial plugs of *F. oxysporum* were placed 0.5 cm from the growing plant root tip in a Petri dish. The plates were incubated at room temperature. Seedlings were examined daily for infected plants, i.e., these exhibited discoloration of the growing root tissues. Infected seedlings were sampled and used to

re-isolate *F. oxysporum* (Koch's postulates). The re-isolation was carried out as explained in Sect. 2.2.

The growth chamber test was conducted on seedlings kept at 24 ± 1 °C and a relative humidity of 55 % and with a 12-h photoperiod under fluorescent light with a photosynthetic photon flux density (PPFD) of $150 \mu\text{mol m}^{-2} \text{s}^{-1}$ at the top of the plant shoots. Seedlings at the 3–4 leaf stage were either (1) inoculated by introducing 1 ml suspension containing 10^6 conidia/ml into a 10-mm cut in the soil near the young stem, or (2) inoculated by placing 5-mm-diameter mycelial plugs of the fungus about 3–5 mm from the stem. Control seedlings were inoculated with 1 ml sterile distilled water. The percent of infected plants was calculated 21 days after inoculation.

In the greenhouse experiment, seedlings were inoculated with mycelial plugs as described above and kept in the greenhouse at 30 °C for 21 days. Twelve replicates were used for each treatment.

2.8 Statistical analysis

Data on the number of infected plants and plant mortality were analyzed using analysis of variance (ANOVA) with the MSTAT-C statistical package (Michigan State University, USA). Prior to analysis, data in percentages were arcsine transformed to correct for the uneven distribution or homogeneity of variance using Excel 2010 (Microsoft, USA). The error of fungal counts was calculated as the standard error of the mean.

3 Results

3.1 Fungal counts and isolates

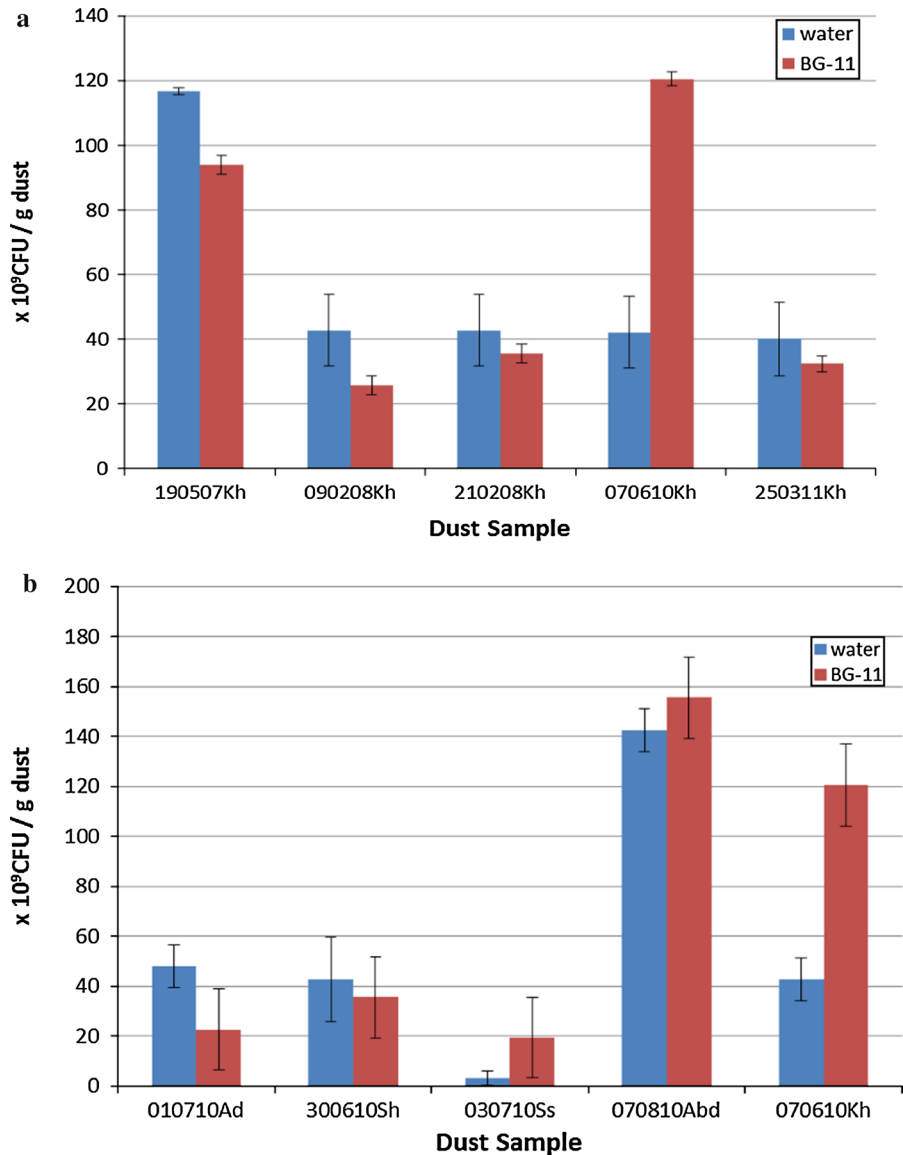
Although most dust samples collected from Khaldiya area showed slightly higher fungal counts when preincubated in water than in BG-11 medium (Fig. 2a), it was the 070610Kh dust sample preincubated in the BG-11 medium that contained the highest fungal count among all Khaldiya dust samples. Among the dust samples collected in 2010, the highest fungal counts were obtained in samples from the Abdely farm area (070810Abd), especially when cultured using BG-11 medium (Fig. 2b). The lowest fungal counts were obtained from the samples collected from the Sabah Al-Salem area (030710Ss).

A total of 228 fungal strains were isolated from the different dust suspensions. The cultures which exhibited different growth qualities were variable in colors (whitish to black), and their textures were smooth, pionnotal or fluffy. Based on their morphologies and cultural traits, these fungal cultures were sorted into 13 genera: *Fusarium* (23.2 % of the total isolates), *Alternaria* (19.7 %), *Ulocladium* (13.6 %), *Phoma* (11.8 %), *Aspergillus* (8.8 %), *Acremonium* (7.7 %), *Penicillium* (7.02 %), *Chaetomium* (2.6 %), *Phaeosphaeria* (0.44 %), *Trichoderma* (0.44 %), *Cladosporium* (0.44 %), *Botryosphaeria* (0.44 %) and *Rhizopus* (0.44 %). Cultures that did not fit in any of these groups were placed in the separate group 14 (3.9 %). For further studies, a total of 51 isolates were randomly selected from all 14 groups. They were examined microscopically for morphological features, such as the spore type, shape, ornamentation and mycelia. The phylogenetic information for 45 out of the 51 isolates is presented in Table 2. Sequence identities between the isolates and sequences in the GenBank database were between 100 and 97 %. Sequences were deposited in the GenBank database (accession numbers: JX885774–JX885818). Isolates of group 14 were affiliated with *Cordyceps* sp., *Preussia* sp., *Emericella* sp. and *Lasiobolus* sp. The sequences of *Aspergillus* sp. retrieved sequence identities in BLAST as either *Aspergillus* sp. or *Emericella* sp. depending on the reproductive phase. Fungi associated with the different dust samples were affiliated with *Fusarium* sp., *Penicillium* sp., *Aspergillus* sp., *Chaetomium* sp., *Phoma* sp., *Alternaria* sp., *Botryosphaeria* sp., *Lasiobolus* sp., *Phaeosphaeria* sp., *Emericella* sp., *Cordyceps* sp., *Ulocladium* sp., *Hypocrea* sp., *Eremodonthis* sp., *Preussia* sp., *Cladosporium* sp. and *Amylomyces* or *Rhizopus* sp.

The diversity of the fungal taxa in each dust sample is shown in Fig. 3. The highest diversity was found in the Khaldiya sample collected in February 9, 2008 (090208Kh). The lowest diversity was found in dust samples collected from Shamiyah (300610Sh) and Sabah Al-Salem (030710Ss).

Abdely samples (070810Abd) had the highest number of fungi which have been affiliated with *Alternaria* sp., *Ulocladium* sp., *Aspergillus* sp., *Fusarium* sp., *Phoma* sp. and *Eremodonthis* sp. In addition, *Cladosporium* sp., *Cordyceps* sp., *Chaetomium* sp., *Phaeosphaeria* sp., *Botryosphaeria* sp., *Hypocrea* sp., *Amylomyces* sp. and *Preussia* sp. were the least

Fig. 2 Comparison of fungal CFU numbers per gram of dust sample collected in **a** Khaldiya (Kh) during five separate dust storm events and **b** from different dust storms in different areas during 2010. *Ad* Adan, *Sh* Shamiyah, *Ss* Sabah Al-Salem, *Abd* Abdely, *Kh* Khaldiya. Error bars represent standard error



abundant isolates and were only found in a few dust samples.

The fungal taxa in Fig. 3 included saprotrophic soil fungi and potential plant pathogens. The latter comprised, e.g., *Alternaria alternata*, *Aspergillus* sp., *Botryosphaeria* sp., *Cladosporium* sp., *F. oxysporum* strains, *F. moniliforme*, *Penicillium* spp., *Phoma* sp. and *Rhizopus stolonifera* (Parry 1990). The Abdely dust sample had the largest number of potential plant pathogens.

The pathogenicity tests were done on *Fusarium* isolates only. Mycelia of such isolates on PDA were initially colorless, turned cream or buff and eventually

reddish to pink or purple at 25 °C and under fluorescent light. These cultures produced both microconidia and macroconidia. Microconidia were mostly unicellular, rarely bicellular. Macroconidia, which were carried on branched conidiophores, were slightly curved and consisted of 3–5 cells each. As the cultures aged, they developed terminal or intercalary chlamydospores.

3.2 Pathogenic isolates

In the laboratory bioassay, tomato, cucumber and bean, but not squash seedlings, were successfully

Table 2 Fungal isolates originating from different dust samples, showing the nearest GenBank match, similarity percentage and accession number for sequenced partial 18S rRNA genes of each isolate

No.	Dust sample-medium ^a	Nearest match in GenBank (accession no.)	Identity %	Isolate name and KUFC ^b	Isolate accession no.
1	090208Kh-W	<i>Fusarium oxysporum</i> strain MZ10-7 (JQ281529.1)	99	<i>Fusarium oxysporum</i> KUFC-D-28	JX885774
2	210208Kh-W	<i>Fusarium oxysporum</i> strain MZ10-7 (JQ281529.1)	100	<i>Fusarium oxysporum</i> KUFC-D-15	JX885782
3	070610Kh-M	<i>Fusarium oxysporum</i> strain MZ10-7 (JQ281529.1)	99	<i>Fusarium oxysporum</i> KUFC-D-122	JX885787
4	090208Kh-M	<i>Fusarium oxysporum</i> strain MZ3-3 (JQ256471.1)	99	<i>Fusarium oxysporum</i> KUFC-D-159	JX885818
5	070610Kh-M	<i>Fusarium oxysporum</i> strain MZ10-7 (JQ281529.1)	100	<i>Fusarium oxysporum</i> KUFC-D-98	JX885794
6	210208Kh-W	<i>Aspergillus terreus</i> strain IG4 (JQ012802.1)	100	<i>Aspergillus terreus</i> KUFC-D-20	JX885777
7	070610Kh-M	<i>Aspergillus awamori</i> strain RU5 (JQ256463.1)	100	<i>Aspergillus terreus</i> KUFC-D-44	JX885801
8	210208Kh-W	<i>Aspergillus fumigatus</i> strain SK1 (JQ665711.1)	100	<i>Aspergillus fumigatus</i> KUFC-D-29	JX885784
9	190507Kh-W	<i>Aspergillus fumigatus</i> strain SK1 (JQ665711.1)	100	<i>Aspergillus fumigatus</i> KUFC-D-04	JX885789
10	250311KH-M	<i>Chaetomium globosum</i> isolate W7 (JQ686920.1)	100	<i>Chaetomium globosum</i> KUFC-D-300	JX885803
11	070610Kh-W	<i>Chaetomium globosum</i> isolate W7 (JQ686920.1)	99	<i>Chaetomium globosum</i> KUFC-D-43	JX885793
12	090208Kh-M	<i>Chaetomium globosum</i> isolate W7 (JQ686920.1)	100	<i>Chaetomium globosum</i> KUFC-D-155	JX885816
13	190507Kh-M	<i>Chaetomium globosum</i> isolate W7 (JQ686920.1)	99	<i>Chaetomium globosum</i> KUFC-D-144	JX885778
14	070610Kh-M	<i>Phoma</i> sp. F40 (JQ390156.1)	100	<i>Phoma</i> sp. KUFC-D-47	JX885783
15	090208Kh-M	<i>Phoma</i> sp. F40 (JQ390156.1)	100	<i>Phoma</i> sp. KUFC-D-130	JX885791
16	090208Kh-M	<i>Phoma</i> sp. F40 (JQ390156.1)	100	<i>Phoma</i> sp. KUFC-D-154	JX885796
17	210208Kh-M	<i>Phoma</i> sp. F40 (JQ390156.1)	99	<i>Phoma</i> sp. KUFC-D-58	JX885799
18	070810Abd-M	<i>Phoma</i> sp. F40 (JQ390156.1)	100	<i>Phoma</i> sp. KUFC-D-303	JX885806
19	210208Kh-W	<i>Phoma</i> sp. F40 (JQ390156.1)	100	<i>Phoma</i> sp. KUFC-D-106	JX885776
20	070810Abd-M	<i>Phoma</i> sp. F40 (JQ390156.1)	100	<i>Phoma</i> sp. KUFC-D-304	JX885807
21	190507Kh-M	<i>Alternaria</i> sp. F38 (JQ390154.1)	99	<i>Alternaria</i> sp. KUFC-D-68	JX885779
22	190507Kh-M	<i>Alternaria</i> sp. F38 (JQ390154.1)	99	<i>Alternaria</i> sp. KUFC-D-65	JX885780
23	210208Kh-M	<i>Alternaria</i> sp. F38 (JQ390154.1)	100	<i>Alternaria</i> sp. KUFC-D-108	JX885781
24	190507Kh-W	<i>Alternaria</i> sp. F38 (JQ390154.1)	100	<i>Alternaria</i> sp. KUFC-D-03	JX885802
25	300610Sh-W	<i>Alternaria</i> sp. F38 (JQ390154.1)	100	<i>Alternaria</i> sp. KUFC-D-141	JX885817
26	090208Kh-M	<i>Penicillium chrysogenum</i> strain D5 (JF718786.1)	100	<i>Penicillium chrysogenum</i> KUFC-D-55	JX885775
27	070610Kh-W	<i>Penicillium decumbens</i> strain lz-13 (JQ664732.1)	98	<i>Penicillium decumbens</i> KUFC-D-78	JX885785
28	190507Kh-M	<i>Penicillium decumbens</i> strain lz-13 (JQ664732.1)	100	<i>Penicillium decumbens</i> KUFC-D-135	JX885786
29	090208Kh-M	<i>Phaeosphaeria</i> sp. YK31 (JN5446133.1)	100	<i>Phaeosphaeria</i> sp. KUFC-D-35	JX885792
30	190507Kh-M	<i>Ulocladium botrytis</i> UPSC 3539 (AF548106.1)	100	<i>Ulocladium botrytis</i> KUFC-D-66	JX885815
31	090208Kh-M	<i>Ulocladium botrytis</i> UPSC 3539 (AF548106.1)	98	<i>Ulocladium botrytis</i> KUFC-D-156	JX885812
32	190507Kh-M	<i>Ulocladium botrytis</i> UPSC 3539 (AF548106.1)	99	<i>Ulocladium botrytis</i> KUFC-D-06	JX885800
33	190507Kh-M	<i>Ulocladium botrytis</i> UPSC 3539 (AF548106.1)	97	<i>Ulocladium botrytis</i> KUFC-D-71	JX885813

Table 2 continued

No.	Dust sample-medium ^a	Nearest match in GenBank (accession no.)	Identity %	Isolate name and KUFC ^b	Isolate accession no.
34	070610Kh-W	<i>Hypocrea lixii</i> (JQ806366.1)	100	<i>Hypocrea lixii</i> KUFC-D-116	JX885811
35	070810Abd-M	<i>Cladosporium</i> sp. WC6 (JN040749.1)	100	<i>Cladosporium</i> sp. KUFC-D-306	JX885809
36	090208Kh-M	<i>Botryosphaeria laricina</i> gene for (AB454206.1)	100	<i>Botryosphaeria laricina</i> KUFC-D-61	JX885788
37	210208Kh-W	<i>Eremodothis angulata</i> CBS 610.74 (DQ384067.1)	100	<i>Eremodothis angulata</i> KUFC-D-105	JX885795
38	210208Kh-M	<i>Eremodothis angulata</i> CBS 610.74 (DQ384067.1)	100	<i>Eremodothis angulata</i> KUFC-D-125	JX885798
39	210208Kh-W	<i>Eremodothis angulata</i> CBS 610.74 (DQ384067.1)	99	<i>Eremodothis angulata</i> KUFC-D-10	JX885814
40	070610Kh-M	<i>Amylomyces rouxii</i> strain PAMF7 (JN256016.1)	100	<i>Amylomyces rouxii</i> KUFC-D-307	JX885810
41	070610Kh-M	<i>Fusarium</i> sp. F19 (JQ390151.1)	100	<i>Fusarium</i> sp. KUFC-D-120	JX885797
42	070810Abd-M	<i>Preussia</i> sp. CCF3831 (FJ430777.1)	100	<i>Preussia</i> sp. KUFC-D-305	JX885808
43	070810Abd-M	<i>Emicella nidulans</i> (AB008403.1)	100	<i>Emicella nidulans</i> KUFC-D-301	JX885804
44	070810Abd-M	<i>Emicella nidulans</i> (AB008403.1)	100	<i>Emicella nidulans</i> KUFC-D-302	JX885805
45	030710Ss-M	<i>Lasiobolus ciliatus</i> (DQ646532.1)	99	<i>Lasiobolus ciliatus</i> KUFC-D-51	JX885790

KUFC Kuwait University Fungal Culture

^a Growth media used where W = water and M = BG-11 mineral medium

infected with *F. oxysporum* isolated from dust collected from three different locations. Symptoms spread upwards to the hypocotyl or to the developing young shoot, resulting in the seedlings death 7 days after infection. *F. oxysporum* was re-isolated from all the infected seedlings. There was a significant difference in the plants susceptibility to infection by *F. oxysporum* isolates ($P = 0.018$; Fig. 4). In general, bean seedlings were the most susceptible to infection (70–100 %). Cucumber seedlings were the least susceptible to isolate Sh-2010 (12 %), while a Sabah Al-Salem isolate (Ss-2010) was the least virulent to tomato seedlings (22 %).

Under the growth chamber conditions, seedling mortality (percentage of death of infected plants) was significantly different using different *F. oxysporum* isolates ($P = 0.045$; Fig. 5). Although the Kh-2010 isolate successfully infected all bean plants (Fig. 4), this infection was associated with the lowest mortality rate (Fig. 5). There was significantly higher percentage of tomato seedling infected by the mycelia than by the spores ($P = 0.0028$; Fig. 6).

In the greenhouse experiment, there was a significant difference in host susceptibility to the different fungal isolates ($P = 0.005$) with bean seedlings having the highest infection rate (Fig. 7). The isolates Sh-2010 and Ss-2010 were most virulent to tomato (65 %) and bean (90 %) seedlings, respectively. Cucumber seedlings were least susceptible to the Sh-2010 isolate (13 %). The initial symptom of infection was expressed in stunted growth and slightly chlorotic and drooping leaves (Fig. 8a, b). There was a significant difference ($P = 0.018$) in plant mortality among the infected host plants (Fig. 9). The highest mortality in tomato plants was in those infected with Kh-2010, but in bean and cucumber, isolates Ss-2010 and Sh-2010, respectively, caused the highest plant mortality rates.

4 Discussion

The results of this study offer experimental evidence that the northwesterly dust storms in Kuwait transport saprophytic and potential plant pathogenic fungi as viable propagules. The most common dust-carried fungi belonged to the genera *Alternaria*, *Ulocladium*, *Aspergillus*, *Penicillium*, *Fusarium*, *Acremonium* and *Phoma*.

Fig. 3 Percentages of different fungal genera in each dust sample collected from Khaldiya (190507Kh, 210208Kh, 090208Kh, 070610Kh and 250311Kh); Sabah Al-Salem (030710Ss); Adan (010710Ad); Shamiyah (300610Sh); and Abdely (070810Abd)

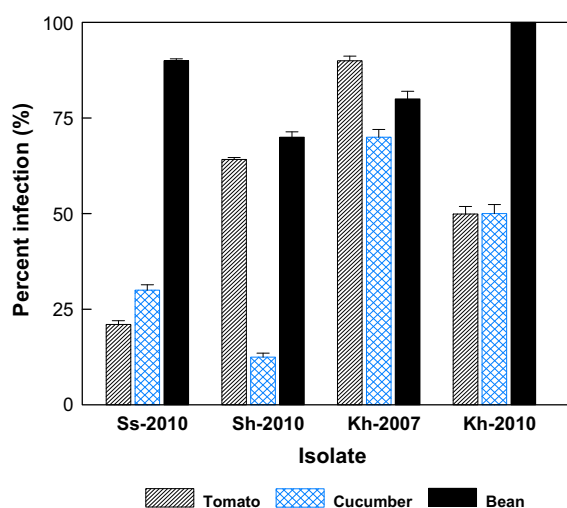
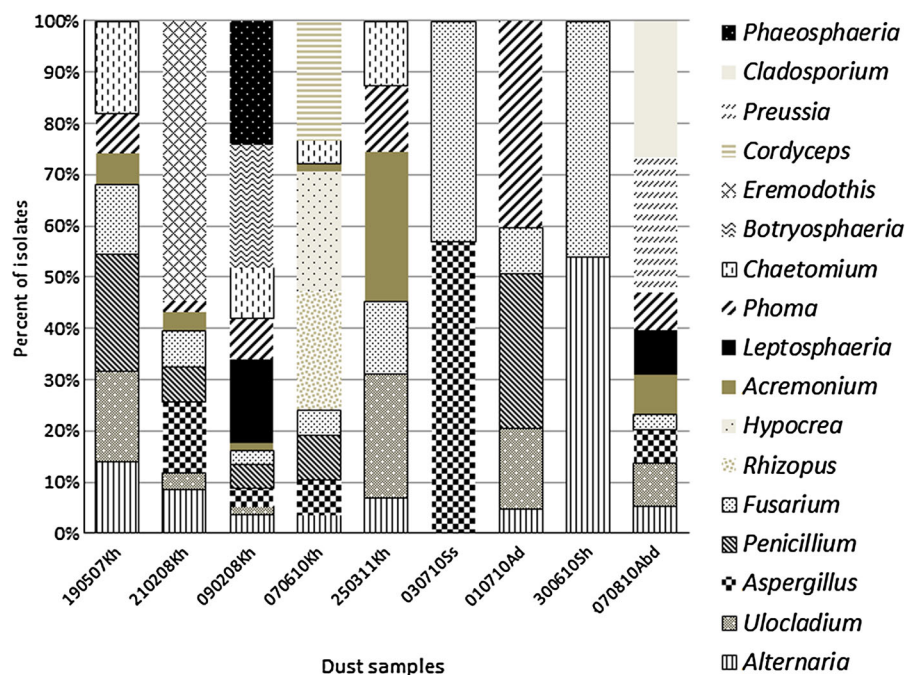


Fig. 4 Percent of infection in a laboratory bioassay of tomato, cucumber and bean seedlings. A total of 144 plants were inoculated with different, dust-isolated *F. oxysporum*. Error bars represent the standard error of the mean

Although preincubation in water was adequate to bring fungi to grow, BG-11 medium supported the highest counts. The difference in fungal counts in water and the BG-11 medium is in part due to the ability of each fungal strain to thrive in the medium used. This observation is in agreement with the

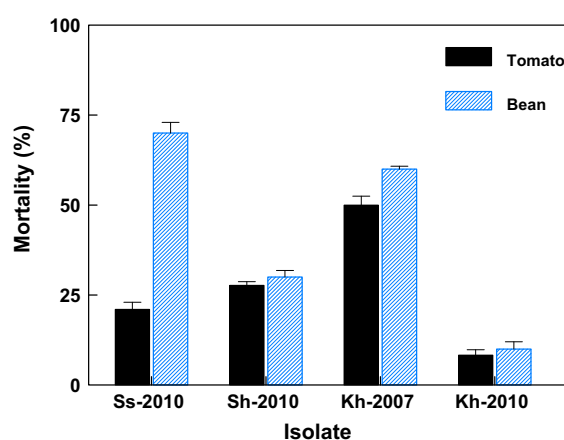


Fig. 5 The plant mortality percent in growth chamber incubated seedlings of tomato, cucumber and bean caused by different isolates of *F. oxysporum*. Sample size is 12 seedlings for each plant type and fungal treatment. Error bars represent the standard error of the mean

findings of Abdel-Hafez and Shoreit (1985). The mineral-based BG-11 medium was previously reported to be suitable for growth of fungi of different origins (Gaylarde and Gaylarde 1999).

Studies of pathogenic microbes in dust storms or settled dust storms are relatively few compared to those conducted on pathogens on normal air and soil

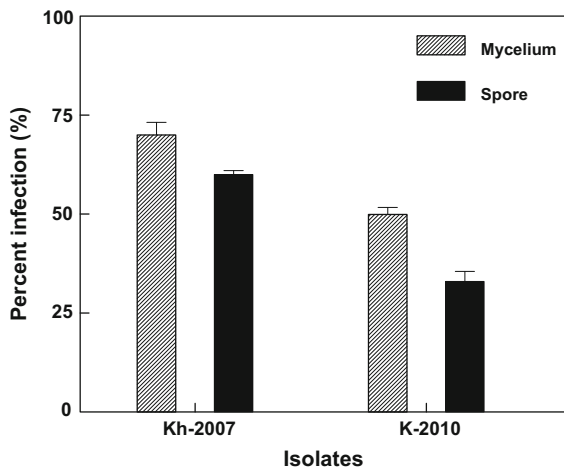


Fig. 6 The percent infection of total of 48 tomato plants caused by two dust isolates of *F. oxysporum* inoculated in the form of either spores or mycelia plugs. Error bars represent the standard error of the mean

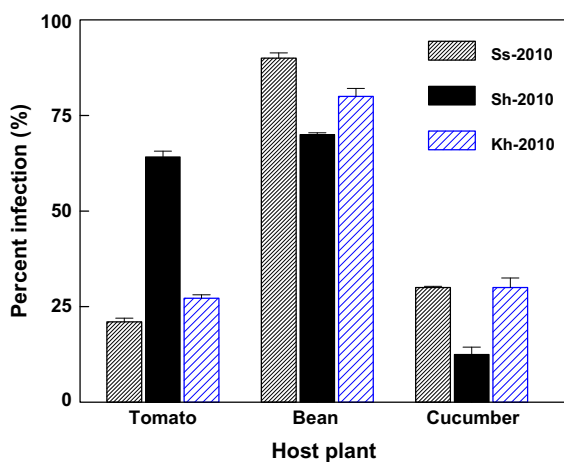


Fig. 7 The virulence of three isolates of *F. oxysporum* expressed as the percent of infection in a sample size of 12 seedlings of greenhouse grown tomato, bean and cucumber plants. The fungal isolate Ss-2010 was obtained from dust collected from Sabah Al-Salem; Sh-2010 from Shamiyah and Kh-2010 from Khaldiya. Error bars represent the standard error of the mean

environments (e.g., Abdel-Hafez et al. 1986; Abdel-Hafez and Shoreit 1985; Kellogg et al. 2004; Griffin 2007; Hara and Zhang 2012). Most studies on fungal pathogens in dust storms have put emphasis on human and, to some extent, animal pathogens (e.g., Bener

et al. 1996; Al Frayh et al. 2001). Very few studies have been conducted so far on plant pathogens load in dust storms. This rarity highlights the novelty and value of the current study. According to the few previous reports, dust storms, as well as desert soils, may harbor pathogenic and non-pathogenic fungi (Kellogg et al. 2004). Some of these fungi, e.g., *Acremonium* sp., *Alternaria* sp., *Aspergillus* sp., *Fusarium* sp., *Phoma* sp., *Penicillium* sp. and *Cladosporium* sp., are mild to potent allergens (Griffin 2007). Others are “opportunistic” plant pathogens or facultative plant parasites.

Fusarium is a typical soil and rhizosphere inhabitant. About 80–90 % of fungal mycobiota in the rhizosphere of several agricultural crops contain *Fusarium* species (Elias et al. 1991). Therefore, dust storms passing over agricultural lands are likely to carry *Fusarium* propagules. Strains of this fungus show some degree of host specificity. Strains of *F. oxysporum* were classified into formae speciales on the basis of their specific host. For example,



Fig. 8 The effect of *F. oxysporum* **a** on tomato plants and **b** cucumber plants. Plants incubated with fungal isolates are on the right and the control plants are on the left

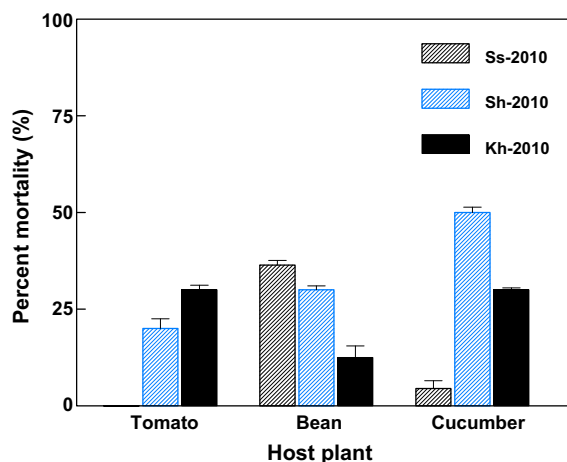


Fig. 9 The virulence of different *F. oxysporum* isolates expressed as the percent of plant mortality. The isolates were obtained from dusts collected from three separate locations in 2010. Ss-2010 was from Sabah Al-Salem; Sh-2010 was from Shamiyah and Kh-2010 was from Khaldiya. Sample size was 12 for each experiment. Error bars represent the standard error of the mean

F. oxysporum f. sp. *Lycopersici* is specifically pathogenic to tomato plants, whereas *F. oxysporum* f. sp. *cucumerinum* is pathogenic to cucumber plants (Snyder and Hansen 1940). However, strains of *F. oxysporum*, whether pathogenic, nonpathogenic or a different formae speciales, are morphologically indistinguishable (Puhalla 1984; Fravel et al. 2003).

In this study, identification of the different strains of *Fusarium* isolates was attempted, but the 18S ribosomal RNA gene sequencing apparently was not specific enough for this identification. This study showed that the dust samples contained propagules of strains of *Fusarium* that were pathogenic to tomato, bean and cucumber plants, but not to squash plants. This observation implies that the *F. oxysporum* isolates have a broad host range. Some formae speciales have broad host ranges, e.g., *F. oxysporum* f. sp. *radicis-lycopersici* and *F. oxysporum* f. sp. *radicis-cucumerinum*. They not only infect tomato and cucumber plants, respectively, but also can cause root and stem rot to a number of host plants from different families (Rowe 1980; Menzies et al. 1990; Vakalounakis 1996; Lievens et al. 2008).

In conclusion, plant pathogenic fungi as soil inhabitants or soil invaders can be transported alive by dust storms and have the potential to cause disease to susceptible host plants. The socioeconomic aspects of dust storms need to be given more attention and

should include the impact of dust storms on agricultural crops and vegetation of the region. The fungal isolates in this study represent only a small fraction of a larger microflora. Future studies using DNA isolated directly from dust samples may provide more accurate information regarding both the culturable and the non-culturable fungi in dust storms.

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