

## Soil mycobiota of date palm plantations in Elche, SE Spain

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The mycobiota of soil from date palm (*Phoenix dactylifera*) plantations in Elche, SE Spain was examined using 23 soil samples and five isolation methods. One hundred and nineteen species assigned to 67 genera were isolated. The most frequent species were in decreasing order: *Aspergillus fumigatus*, *A. niger*, *Neosartorya spinosa*, *Thielaviopsis punctulata*, *Chaetomium bostrychodes*, *Gilmaniella macrospora*, *Aspergillus candidus*, *Fusarium oxysporum*, *Rhizopus microsporus*, *Sordaria fimicola*, *Aspergillus terreus*, *Chaetomium murorum*, *Fusarium solani*, *Mucor racemosus*, *Penicillium citrinum* and *Thielaviopsis paradoxa*. The thermotolerant and thermophilic species of *Malbranchea cinnamomea*, *Myriococcum thermophilum*, *Rhizomucor miehei*, *Scytalidium thermophilum*, *Talaromyces emersonii*, *Thermoascus aurantiacus* and *Thermomyces lanuginosus* were detected in various frequencies of occurrence. Our findings are compared with those from a similar survey of soil from date palm plantations in Iraq. Our study indicates that there is no characteristic mycobiota for soil in date palm plantations except for the more frequent isolation of some species well known as pathogens on date palm.

**Key words:** soil saprotrophic fungi, phytopathogenic fungi, *Phoenix dactylifera*, isolation methods.

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Půdní mykobiota datlovníkové plantáže (*Phoenix dactylifera*) u Elche v jv. Španělsku byla studována ve 23 půdních vzorcích a pomocí pěti izolačních metod. Bylo izolováno 119 druhů z 67 rodů. Nejčastější druhy jsou uvedeny v anglickém abstraktu, kde jsou zmíněny i některé termotolerantní a termofilní druhy. Výsledky jsou srovnány s podobnou studií z Iráku. Ukázalo se, že datlovníkové plantáže nemají specifickou mykobiotu s výjimkou častějšího zastoupení patogenů datlovníku.

### INTRODUCTION

The soil mycobiota of date palm (*Phoenix dactylifera* L.) plantations has received little attention in spite of the vast areas dominated by date palm groves in several countries in the Middle East and North Africa. However, recent investigations on soil fungi of several date palm plantations in Iraq have revealed several

novel and interesting fungal taxa (Abdullah and Zora 1993 a, b; Horie et al. 1990; Udagawa et al. 1985, 1986). Date palm is native in North Africa. It was introduced in Southern Spain and is nowadays widely distributed throughout the country forming extensive palm groves. The only two palm groves with commercial value in Europe are located in South East Spain, namely in Elche and Orihuela. The „Palmeral“ (palm forest or palm grove) of Elche, known as „Palmeral of Europe“, was declared a World Heritage by UNESCO in 2000. The soil mycobiota of date palm plantations has never been studied in Spain. The present investigation was carried out on soil fungi present in a palm grove in Elche to study species distribution, diversity and taxonomy, and to compare these data with those obtained from palm groves in Iraq.

#### MATERIALS AND METHODS

**Study area.** Elche grove is located in Alicante Province, South East Spain very close to the Mediterranean coast (0° 47' 30" W, 39° 9'–38° 21' N). It consists of several scattered date palm plantations with genetically different trees. Elche palm grove is up to 300 years old. The number of adult date palm plants is estimated to be around 200.000 individuals (Orts and Lopez-Jimenes 2003) and the total area of the palm grove is about 400 hectares (Ferry and Greiner 1997). The average monthly temperature ranges from 11.2 to 26.6 °C and the mean annual rainfall is approximately 286 mm. The soil is loamy sand.

**Soil collection.** Twenty-three soil samples were collected in several date palm plantations at Elche during May–June, 2004. Approximately 500 g of soil was removed with a sterile trowel from a depth of 2–10 cm at each site after first removing the upper 0–2 cm of surface soil. Soil samples were taken from neglected areas with no associated crops. Soil samples were stored in polythene bags at 5 °C in the dark and were processed within 1–2 weeks after collection.

**Isolation of fungi.** Five isolation methods were used: suspension plating method (Bills et al. 2004), direct plating method (Warcup 1960), soil treatment with 5 % acetic acid (Furuya and Naito 1979), treatment with 2 % phenol (Furuya and Naito 1980) and treatment with 70 % ethanol (Warcup and Baker 1960). Three types of media were used to isolate fungi, viz. potato carrot agar (PCA) (20 g peeled potatoes, 20 g carrot, 20 g agar, 1 l distilled water), malt extract agar (MEA, Scharlau, Barcelona, Spain), and potato dextrose agar (PDA, Oxoid, England). Each medium was supplemented with 50 µg·ml<sup>-1</sup> chloramphenicol (Sigma, USA) to suppress bacterial growth. Plates for all methods and media (six replicates each) were incubated at 25 and 40 °C in the dark. Single colonies were picked from the plates under a dissecting microscope and transferred to appropriate media to allow fungus development. Single spore cultures were not used in this study.

Identification of fungi. General and specific taxonomic literature was used for the identification of fungal species: Arx et al. (1986), Bell (1983), Cannon and Hawksworth (1982, 1984), Coony and Emerson (1964), Domsch et al. (1993), Ellis (1971, 1976), Klich (2002), Mouchacca (1997), Moustafa (1975), Paulin-Mahady et al. (2002), Samson et al. (2000), and Sutton (1980). Representative strains of the fungal species were deposited at the culture collection of the Marine and Applied Biology Department, Alicante University, Spain.

## RESULTS

One hundred and nineteen fungal species representing 67 genera were isolated from 23 soil samples collected from Elche palm grove. Their frequency of occurrence is presented in Tab. 1. The fungi isolated have been divided into four groups according to their occurrence: H = high frequency (> 50 %), M = moderate frequency (25 to 50 %), L = low frequency (12 to 25 %), VL = very low frequency (< 12 %).

Sixteen species were isolated with high frequencies (listed in decreasing order): *Aspergillus fumigatus*, *A. niger*, *Neosartorya spinosa*, *Thielaviopsis punctulata*, *Chaetomium bostrychodes*, *Gilmaniella macrospora*, *Aspergillus candidus*, *Fusarium oxysporum*, *Rhizopus microsporus*, *Sordaria fimicola*, *Aspergillus terreus*, *Chaetomium murorum*, *Fusarium solani*, *Mucor racemosus*, *Penicillium citrinum*, and *Thielaviopsis paradoxa*. The first four species occurred in 100 % of the soil samples. The group with moderate frequencies of isolation included 27 fungal species, while that with low frequencies was represented by 39 species. The remaining 38 fungal species were isolated at very low frequencies (Tab. 1).

*Aspergillus* was represented by 11 species and thus showed the widest diversity among all recovered genera. *A. fumigatus* and *A. niger* were the most frequent species within the genus. *Penicillium* was second in the number of species isolated and was represented by nine species. *P. citrinum* was the most frequent, occurring in 52 % of the cases. Six species of the genus *Fusarium* were identified. Among these *F. oxysporum* and *F. solani* were the most common with a frequency of 61 % and 57 %, respectively (Tab. 1).

Anamorphic hyphomycetes other than Aspergilli, Penicillia and Fusaria, were represented by 48 species distributed over 33 genera. Among them *Thielaviopsis punctulata*, *T. paradoxa* and *Gilmaniella macrospora* belonged to the high frequency group, while taxa identified as *Acremonium strictum*, *Alternaria alternata*, *Scopulariopsis candida*, *Stachybotrys* sp., *Trichoderma hamatum*, *T. harzianum*, *Thermomyces lanuginosus*, *Trichurus spiralis* and *Myrothecium verrucaria* showed a moderate occurrence.

**Tab. 1.** List of fungi isolated from soil of date palm plantations at Elche (SE Spain) with five isolation methods and their relative frequency of occurrence.

Fungi	Frequency (%) <sup>1</sup>	Frequency group	Method
<i>Acremonium kiliense</i> Grütz	8.7	VL	Ac P
<i>A. strictum</i> W. Gams	26.0	M	D Ph P
<i>Alternaria alternata</i> (Fr.) Keissl.	34.7	M	Ac P
<i>A. chlamydospora</i> Mouch.	4.3	VL	P
<i>Amerosporium polynematoides</i> Speg.	4.3	VL	P
<i>Ascodesmis nigricans</i> Tiegh.	4.3	VL	E
<i>Aspergillus candidus</i> Link	60.8	H	Ac D Ph P
<i>A. carbonarius</i> (Bainier) Thom	8.7	VL	Ph
<i>A. carneus</i> (Tiegh.) Blochwitz	4.3	VL	P
<i>A. flavus</i> Link	8.7	VL	Ac P
** <i>A. fumigatus</i> Fresen.	100.0	H	Ac D E Ph P
<i>A. melleus</i> Yukawa	4.3	VL	D
<i>A. niger</i> Tiegh.	100.0	H	Ac D E Ph P
<i>A. niveus</i> Blochwitz	8.7	VL	Ph
<i>A. ochraceus</i> K. Willh.	13.0	L	E P
<i>A. terreus</i> Thom	56.5	H	Ac D E Ph P
<i>A. ustus</i> (Bainier) Thom & Church	4.3	VL	P
<i>Auxarthron umbrinum</i> (Boud.) G.F. Orr & Plunkett	8.7	VL	Ph
<i>Beauveria bassiana</i> (Bals.-Criv.) Vuill.	21.7	L	D P
<i>Chaetomidium fimeti</i> (Fuckel) Sacc.	13.0	L	Ph
<i>Chaetomium atrobrunneum</i> L.M. Ames	13.0	L	D P
<i>C. bostrychodes</i> Zopf	73.9	H	Ac D Ph P
<i>C. elatum</i> Kunze	17.4	L	P
<i>C. megalocarpum</i> Bainier	21.7	L	P
<i>C. murorum</i> Corda	56.5	H	Ac D Ph P
<i>Cladorrhinum foecundissimum</i> Sacc.	4.3	VL	P
<i>Cladosporium cladosporioides</i> (Fresen.) G.A. de Vries	17.4	L	P
<i>C. herbarum</i> (Pers.) Link	8.7	VL	P
<i>Clonostachys rosea</i> (Link) Schroers et al.	17.4	L	E
<i>Colletotrichum</i> sp.	4.3	VL	P
** <i>Corynascus sepedonium</i> (C.W. Emmons) Arx	26.0	M	Ph P
** <i>Corynascus</i> sp.	4.3	VL	P
<i>Cunninghamella echinulata</i> (Thaxt.) Thaxt.	26.0	M	Ac P
<i>Doratomyces microsporus</i> (Sacc.) F.J. Morton & G. Sm.	8.7	VL	Ph
<i>Emericella nidulans</i> (Eidam) Vuill.	34.7	M	Ac P
<i>E. rugulosa</i> C.R. Benj.	17.4	L	P
<i>Emericellopsis terricola</i> J.F.H. Beyma	4.3	VL	Ph
<i>Eupenicillium</i> sp.	21.4	L	E
<i>Eurotium</i> sp.	17.4	L	E
<i>Fusarium equiseti</i> (Corda) Sacc.	13.0	L	D P

Fungi	Frequency (%) <sup>1</sup>	Frequency group	Method
<i>F. oxysporum</i> Schltld.	60.8	H	D P
<i>F. poae</i> Wollenw.	4.3	VL	P
<i>F. redolens</i> Wollenw.	13.0	L	D P
<i>F. solani</i> (Mart.) Sacc. aggr.	56.5	H	D P
<i>F. subglutinans</i> (Wollenw. & Reinking) P.E. Nelson, Toussoun & Marasas	30.4	M	D P
<i>Gilmaniella humicola</i> G.L. Barron	26.0	M	D P
<i>G. macrospora</i> Moustafa	69.5	H	Ac D Ph P
<i>Gliocladium penicillioides</i> Corda	13.0	L	Ph P
<i>Graphium penicillioides</i> Corda	17.4	L	E P
<i>G. putredinis</i> (Corda) S. Hughes	8.7	VL	Ph
<i>Gymnoascus reessii</i> Baran	26.0	M	Ac P
<i>Heteroconium chaetospira</i> (Grove) M.B. Ellis	4.3	VL	P
<i>Humicola fuscoatra</i> Traaen	21.7	L	Ph P
<i>H. grisea</i> Traaen	13.0	L	P
<i>Lasiodiplodia theobromae</i> (Pat.) Griffon & Maubl.	34.7	M	P
* <i>Malbranchea cinnamomea</i> (Lib.) Oorschot & de Hoog	17.4	L	Ph P
<i>Melanospora longisetosa</i> P.F. Cannon & D. Hawksw.	17.4	L	P
<i>M. zamiae</i> Corda	34.7	M	P
<i>Metarhizium anisopliae</i> (Metschn.) Sorok.	13.0	L	P
<i>Microascus cinereus</i> Curzi	21.7	L	Ac D Ph
<i>M. trigonosporus</i> C.W. Emmons & B.O. Dodge	39.1	M	D Ph
** <i>Mycocladus corymbifer</i> (Cohn) Váňová	17.4	L	Ac Ph P
<i>Mucor hiemalis</i> Wehmer	17.4	L	D P
<i>M. racemosus</i> Fresen.	56.5	H	D Ph
* <i>Myriococcum thermophilum</i> (Fergus) Aa	26.0	M	Ac Ph P
<i>Myrothecium roridum</i> Tode	13.0	L	Ac P
<i>M. verrucaria</i> (Alb. & Schwein.) Ditmar	34.7	M	P
<i>Neocosmospora africana</i> Arx	4.3	VL	Ph
** <i>Neosartorya fischeri</i> (Wehmer) Malloch & Cain	43.4	M	E Ph
** <i>N. spinosa</i> (Raper & Fennell) Kozak.	100.0	H	E Ph
<i>Nigrospora sphaerica</i> (Sacc.) E.W. Mason	4.3	VL	P
<i>Paecilomyces variotii</i> Bainier	13.0	L	E P
<i>Penicillium brevicompactum</i> Dierckx	13.0	L	D P
<i>P. chrysogenum</i> Thom	26.0	M	D P
<i>P. citrinum</i> Thom	52.1	H	D E P
<i>P. digitatum</i> (Pers.) Sacc.	13.0	L	D P
<i>P. expansum</i> Link	34.7	M	D E P
<i>P. glabrum</i> (Wehmer) Westling	47.8	M	D E Ph P
<i>P. funiculosum</i> Thom	47.8	M	D E Ph P
<i>P. restrictum</i> J.C. Gilman & E.V. Abbott	30.4	M	E Ph
<i>P. sacculum</i> E. Dale	17.4	L	Ph P

Fungi	Frequency (%) <sup>1</sup>	Frequency group	Method
<i>Periconia macrospinos</i> a Lefebvre & Aar. G. Johnson	8.7	VL	P
<i>Phialophora</i> sp.	4.3	VL	P
<i>Phoma glomerata</i> (Corda) Wollenw. & Hochapfel	4.3	VL	D
<i>P. herbarum</i> Westend.	13.0	L	D P
<i>P. leveillei</i> Boerema & G.J. Bollen	13.0	L	D P
<i>P. putaminum</i> Speg.	4.3	VL	P
<i>Podospora</i> sp.	4.3	VL	Ac
<i>Preussia funiculata</i> (Preuss) Fuckel	4.3	VL	P
* <i>Rhizomucor miehei</i> (Cooney & R. Emers.) Schipper	13.0	L	P
<i>Rhizopus microsporus</i> Tiegh.	60.8	H	Ac D P
<i>R. oryzae</i> Went & Prins. Geerl.	21.7	L	Ac D
<i>R. stolonifer</i> (Ehrenb.) Vuill.	4.3	VL	Ph
<i>Scolecobasidium</i> sp.	4.3	VL	P
<i>Scopulariopsis brumptii</i> Salv.-Duval	13.0	L	Ph P
<i>S. candida</i> Vuill.	30.4	M	Ac D P
<i>S. chartarum</i> (G. Sm.) F.J. Morton & G. Sm.	17.4	L	Ph P
<i>S. fusca</i> Zach	21.7	L	Ac
* <i>Scytalidium thermophilum</i> (Cooney & R. Emers.) Austwick	13.0	L	Ac P
<i>Sordaria fimicola</i> (Roberge ex Desm.) Ces. & De Not.	60.8	H	Ac E Ph P
<i>Stachybotrys chartarum</i> (Ehrenb.) S. Hughes	21.7	L	P
<i>Stachybotrys</i> sp.	47.8	M	Ph P
<i>Stemphylium</i> sp.	4.3	VL	Ac
<i>Taeniolella</i> sp.	4.3	VL	P
* <i>Talaromyces emersonii</i> Stolk	43.4	M	E P
<i>T. flavus</i> (Klöcker) Stolk & Samson	8.7	VL	E
* <i>Thermoascus aurantiacus</i> Miehe	30.4	M	Ph P
* <i>Thermomyces lanuginosus</i> Tsikl.	30.4	M	D Ph P
<i>Thielavia</i> sp.	13.0	L	P
<i>Thielaviopsis paradoxa</i> (de Seynes) Höhn.	52.1	H	Ac D Ph P
<i>T. punctulata</i> (Hennebert) A.E. Paulin, T.C. Harr. & McNew	100.0	H	Ac D Ph P
<i>Torula herbarum</i> (Pers.) Link	8.7	VL	P
<i>Trichoderma hamatum</i> (Bonord.) Bainier	34.7	M	Ph P
<i>T. harzianum</i> Rifai	34.7	M	D Ph P
<i>Trichurus spiralis</i> Hasselbr.	39.1	M	Ac Ph P
<i>Ulocladium chartarum</i> (Preuss) E.G. Simmons	8.7	VL	P
<i>Verticillium</i> sp.	17.4	L	D P
<i>Westerdykella multispora</i> (Saito & Minoura) Cejz & Milko	4.3	VL	P
<i>Zasmidium cellare</i> (Pers.) Fr.	8.7	VL	Ac

1 = Number of positive samples/ total number of samples in which the fungi were detected; \* = incidentally isolated from plates incubated at 40 °C; \*\* = frequently isolated from plates incubated at 40 °C; H = high; M = medium; L = low; VL = very low; Ac = acetic acid treatment; E = ethanol treatment; Ph = phenol treatment; P = direct plating; D = suspension plating method.

*Mucorales* were represented by five genera viz. *Cunninghamella*, *Mycocladius* and *Rhizomucor* (one species each), *Mucor* (two species) and *Rhizopus* (three species). *R. microsporus* and *M. racemosus* fell into the high frequency group.

Coelomycetes were represented by seven species, viz. *Amerosporium polynematoides*, *Colletotrichum* sp., *Lasiodiplodia theobromae*, *Phoma glomerata*, *P. herbarum*, *P. leveillei* and *P. putaminum*. *Lasiodiplodia theobromae* showed a moderate frequency of 35 %. The remaining coelomycetes species occurred at low or very low frequencies.

Thirty-one species of teleomorphic ascomycetes assigned to 21 genera were encountered in this survey (Tab. 1). *Neosartorya spinosa*, *Chaetomium bostrychodes*, *C. murorum* and *Sordaria fimicola* occurred in the high frequency group. *Ascodesmis nigricans*, *Neocosmospora africana*, *Preussia funiculata*, *Podospora* sp. (64 ascospores), *Emericellopsis terricola* and *Westerdykella multisporea* occurred at very low frequencies. The remaining ascomycete species had a moderate to low frequency of isolation.

Thermophilic and thermotolerant fungi were represented by nine species viz. *Rhizomucor miehei*, *Myriococcum thermophilum*, *Scytalidium thermophilum*, *Thermomyces lanuginosus*, *Talaromyces emersonii*, *Thermoascus aurantiacus*, *Malbranchea cinnamomea*, *Corynascus sepedonium*, and *Corynascus* sp.

The Warcup plate method yielded the highest number of species identified (94 species), followed by phenol treatment (43 species). The suspension plating method was third in the number of species isolated (39 species). Acetic acid and ethanol treated soils yielded the lowest number of species (29 and 20, respectively).

## DISCUSSION

### Comparison with palm plantations in Iraq

Our results of fungal isolations from soils in the date palm grove of Elche were compared with those of a similar study carried out in Iraq by Abdullah and Zora (1993). Fifty-two species were common in soil from both Iraq and Spain. There were obvious differences among species composition for genera with darkly pigmented mycelia as well as for ascomycetes. Dematiaceous genera reported from soil in Iraq (Abdullah and Zora 1993) showing a high species diversity were *Alternaria* (five species), *Curvularia* (two species), *Drechslera* (five species) and *Ulocladium* (four species), whereas, *Alternaria* and *Ulocladium* were each represented by two species in the soil at Elche. *Drechslera* and *Curvularia*, two dematiaceous hyphomycetes were frequently encountered in soil in date palm plantations in Iraq, but were not detected in Elche soil. It has been suggested that fungi with darkly pigmented mycelia are well adapted to withstand intense light radiation (Durrell and Shieds 1960, Ranzoni 1968). Abdullah and Al-Bader (1990)

attributed the high diversity among darkly pigmented species in the Iraqi soil to the fact that Iraq is considered as one of the geographic areas receiving the highest solar radiation in the Northern hemisphere (Thalen 1979).

Thirty one species of teleomorphic ascomycetes were recovered during the present survey at Elche, which is approximately the same number of ascomycete species as isolated from soil of date palm plantations in Iraq (Abdullah and Zora 1993). However, the two sites showed differences in their species composition as well as their frequencies of occurrence. For example in the Iraqi soil, *Chaetomium* showed a high diversity (12 species) compared to the Elche soil (5 species). *Chaetomium atrobrunneum*, *C. elatum* and *C. murorum* were common in soils of both locations. None of the ascomycete species detected in soil from Iraq showed a high frequency of occurrence. On the contrary, the two ascomycete species *Neosartorya fischeri* and *N. spinosa* detected in the Elche soil showed moderate and high frequencies of occurrence respectively. This may be attributed to the difference in isolation media or isolation methods which may explain their absence from a site.

### **Influence of isolation methods**

The higher number of teleomorphic ascomycete species identified in the Spanish soil was expected because of the different selective methods employed. Acetic acid, ethanol and phenol have been used effectively to increase the frequency of ascomycete isolations from soil and dung material by stimulating ascospore germination or have a pasteurisation effect on species with thin-walled conidia (Asina and Cain 1977; Bills and Polishook 1993; Furuya and Naito 1979, 1980; Warcup and Baker 1960). Ethanol treatment has an obvious reducing effect in the total number of isolated species. Of 120 fungal species recovered by all methods, 20 species were isolated only after ethanol treatment. Out of these 20 species, eight were ascomycetes. The isolated species were largely assigned to the genera *Eupenicillium*, *Eurotium*, *Neosartorya*, *Talaromyces* and related anamorphic genera of *Aspergillus*, *Penicillium* and *Paecilomyces*. This result is in agreement with previous reports that ethanol treatment favours isolation of *Eurotiales* (Warcup and Baker 1960). The highest number of recovered species (94) recovered by employing the Warcup soil plate method was probably due to the fact that colonies developing on plates were most likely derived from humus particles and hyphae fragments in addition to spores (Warcup 1960).

### **Important genera**

Among the *Aspergilli*, *A. fumigatus*, *A. niger*, *A. candidus* and *A. terreus* were the major species according to their frequency of occurrence. The four species have been reported as the most frequent isolates from soil of date palm plantations in Iraq and among the most frequent isolates from other soils in arid regions



(Abdullah and Zora 1993b; Abdullah et. al. 1986, Gochenaur 1975, Halwagy et al. 1982, Mobasher and Moustafa 1970). This is primarily related to their high tolerance to both relatively high temperatures and drought conditions (Christensen 1969, Durell and Shields 1960, Gochenaur 1975). Abdullah and Al-Bader (1990), working with soil from Iraq, found that the optimum growth of the four species ranged from 35–40 °C, but all of them still showed good growth at 45 °C.

Of the nine *Penicillia* found in our study, *P. citrinum* was the only species of the group of species with a high frequency of isolation, while *P. glabrum* (*P. frequentans* Westling) and *P. funiculosum* were in the group showing moderate frequency of isolation. *Penicillium citrinum* is a cosmopolitan fungus which has been frequently reported from a variety of habitats (Domsch et. al. 1993). The two other species were frequently found as a part of the mycobiota of arid and desert soils (Halwagy et. al. 1982, Moubasher and Moustafa 1970, Moustafa et. al. 1976). *Penicillium brevicompactum* and *P. chrysogenum* were both commonly found in both soils of date palm plantations in both Iraq and Spain. *Gilmaniella macrospora*, *Stachybotrys atra*, *Trichurus spiralis* and *Alternaria alternata* were among the most frequent species of dematiaceous hyphomycetes. *Gilmaniella macrospora* was first described from Kuwait soil (Moustafa 1975), and a subsequently reported from soil of date palm plantations in Basrah, Iraq (Abdullah and Zora 1993b). Abdullah and Al-Bader (1990) have reported the fungus as a thermotolerant species with optimal growth at 45 °C. This most frequently species in our study was found on plates incubated at 45 °C. This fungus apparently represents a new record to the Spanish mycobiota.

Two entomopathogenic fungi, *Beauveria bassiana* and *Metarhizium anisopliae* were isolated at low frequencies. Both species have recently been reported as two of the most frequent insect pathogens in soils from Alicante Province (Asensio et. al. 2003).

The four *Phoma* species, *P. glomerata*, *P. herbarum*, *P. leveillei* and *P. putaminum* are well known ubiquitous soil-borne fungi with a world-wide distribution (Domsch et. al. 1993). *Phoma glomerata* and *P. putaminum* were isolated from various plant species and, are considered to be secondary invaders or opportunistic parasites (Boerema 1993, De Gruyter and Noordeloos 1992).

### **Thermophilic and thermotolerant fungi**

Seven species were only isolated from plates incubated at 40 °C. These include *Rhizomucor miehei*, *Myriococcum thermophilum*, *Scytalidium thermophilum*, *Talaromyces emersonii*, *Thermoascus aurantiacus*, *Thermomyces lanuginosus* and *Malbranchea cinnamomea*. There is a controversy in the literature concerning the precise definition whether a fungus is considered a thermophilic or a thermotolerant (Apinis 1963, Bokhary et. al. 1984, Cooney and Emerson 1964, Evans 1971, Mouchacca 1997). According to Cooney and Emerson (1964), the

above-mentioned seven species are considered thermophilic. However, *Aspergillus fumigatus*, *Corynascus sepedonium*, *Gilmaniella macrospora*, *Neosartorya fischeri* and *N. spinosa* grew most frequently on plates incubated at 40 °C. In a previous study, Abdullah and Al-Bader (1990) have demonstrated that the first three species showed good growth at 45 °C, and should therefore be considered as thermotolerants.

### Exceptions

Some of the isolated species are not true soil fungi viz. *Amerosporium polynematoides*, *Heteroconium chaetospira*, *Zasmidium cellare*, *Scolecobasidium* sp. and *Taeniolella* sp. and may therefore originate from decaying plant material in contact with soil. *Ascodesmis nigricans*, *Podospora* sp., *Preussia funiculata* and *Sordaria fimicola* are common coprophilous fungi. However, they have been repeatedly isolated from soil (Domsch et al. 1993, Furuya and Naito 1979) and perhaps originated from disintegrated dung pellets incorporated into soil. *Cladorrhinum foecundissimum* is a rare fungus isolated at a very low frequency, probably because its conidia are not capable of germination (Arx and Gams 1966). However, the fungus has been isolated from agricultural and forest soils in some European countries ((Domsch et. al. 1993).

### Potentially phytopathogenic fungi

Several soil-borne potentially phytopathogenic fungi were detected during this survey. Species belonging to the genera *Fusarium*, *Lasiodiplodia* and *Thielaviopsis* were the most interesting. Six species of *Fusarium* were identified. *F. oxysporum* and *F. solani* were the most common of them. The two species have also been shown to be the most frequent species isolated from soil of date palm plantations in Iraq (Abdullah and Zora 1993). Several reports have indicated that isolates of the two species were involved in inflorescence rot and root rot diseases of date palm (El-Morsy 1999, Rattan and Al-Dboon 1980). *Lasiodiplodia theobromae* showed a moderate frequency of occurrence. It is well known as a ubiquitous tropical to subtropical plant pathogen as well as a widespread soil-borne saprophyte (Domsch et. al. 1993). The fungus has been reported as a causal agent of several diseases on different parts of date palm trees including bending head, diplodia disease, leaf base rot and inflorescence rot (El-Morsy 1999).

### *Thielaviopsis* species

A notable result from our study was the widespread occurrence of *Thielaviopsis paradoxa* (teleomorph: *Ceratocystis paradoxa* (Dode) C. Moreau) and *T. punctulata* [teleomorph: *Ceratocystis radicolata* (Bliss) C. Moreau] (Paulin-Mahady et. al. 2002) in soil of date palm plantations at Elche. The two species showed a frequency of occurrence of 52.17 % and 100 %, respectively. *Thiela-*

*viopsis paradoxa* and *T. punctulata* have been repeatedly reported as causal agents of several diseases on roots and aerial parts of palms. These species, either alone or in combination, are involved in black scorch of leaves, heart bud rot, bending head, root rot, terminal bud rot and inflorescence rot diseases of date palms (Djerbi 1983, Simone 1993, Suleman et. al. 2002). Unlike the root pathogens, *T. basicola* and *T. thielavioides*, were frequently isolated from field soils by using carrot disks as bait (McIlveen and Edgington 1972, Yarwood 1946). *Thielaviopsis paradoxa* and *T. punctulata* have been mostly isolated from aerial parts of palms (Djerbi 1983, Kile 1993, Simone 1993, Suleman et al. 2001). However, the present study reports the frequent isolation of the two pathogens from soil using the suspension plating method, direct soil plating or by soil treatment either with acetic acid or phenol. Both species are characterised by their development of thick-walled aleuroconidia, either singly (*T. punctulata*) or in chains (*T. paradoxa*) in addition to the phialoconidia. The thick-walled aleuroconidia are likely to play a role as survival propagules in natural soils. The widespread existence of such propagules in these two plant pathogens in soil may possibly infect the newly transplanted offshoots of date palms. It is worthy mentioning that in spite of the occurrence of one or more of the diseases caused by these two pathogens on date palms in Iraq, they have not been isolated from soil in that country (Abdullah and Zora 1993, Al-Doory et al. 1959).

#### CONCLUSIONS

In conclusion, the present study revealed that even if there are some similarities between species composition in the two geographically different regions, there is no characteristic soil mycobiota of date palm plantations except for the more frequent isolation of some species well known as potential pathogens on date palm. The fact that these fungi are not causing obvious disease may indicate the involvement of antagonists in the soil. We are currently testing this hypothesis. Most of the species identified were common typical soil fungi with a worldwide distribution (Domsch et. al. 1993).

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