INCIDENCE OF THE TWO DATE PALM PATHOGENS, *THIELAVIOPSIS PARADOXA* AND *T. PUNCTULATA* IN SOIL FROM DATE PALM PLANTATIONS IN ELX, SOUTH-EAST SPAIN

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Abstract: The present study reports the frequent isolation of the two date palm pathogens *Thielaviopsis paradoxa* (de Seynes) Hohn and *T. punctulata* (Hennebert) Paulin, Harrington et McNew from soil of date palm plantations at Elx, south-east Spain, using dilution plate, direct soil plating or by soil treatment either with acetic acid or phenol. The two species showed a high isolation rate. *T. punctulata* detected from all samples (100% isolation rate), whereas, *T. paradoxa* showed 52% isolation rate.Total fungal colony count, ranged from $1.1x10^5$–$6 x10^5$, CFU/g dry soil. Out of these, *T. punculata* comprised between 0.2–3.2% and *T. paradoxa*, between 0.5–4.4%. Both species were characterized by development of thick-walled aleuroconidia either singly (*T. punctulata*) or in chains (*T. paradoxa*) in addition to the phialoconidia. The widespread occurrence of the two pathogens in soil may contribute to the possibility of infection of newly transplanted offshoots of date palms.

Key words: *Thielaviopsis*, date palm pathogens, soil, Spain

INTRODUCTION

Date palm (*Phoenix dactylifera* L.), a native of North Africa was introduced into Southern Spain and is now widely planted as an ornamental plant grown in parks, gardens and alongside roads. The only two palm groves with commercial value in Europe are located in South East Spain namely in Elx and Orihuela (Abdullah *et al.* 2005).

During a survey of soil mycobiota from date palm plantations in Elx, S.E. Spain (Abdullah *et al*. 2006), we detected a high incidence of two date palm pathogens *Thielaviopsis paradoxa* (de Seynes) Hohn., (Teleomorph: *Ceratocystis paradoxa* (Dade) C. Moreau. and *Thielaviopsis punctulata* (Hennebert) Paulin, Harrington, et McNew., (Teleomorph: *Ceratocystis radicicola* (Bliss) Moreau., in soil and the results are presented here.

MATERIALS AND METHODS

Area of study

Elx grove is located in Alicante province, S.E. Spain very close to the Mediterranean coast (0 47 30 West, 39 9 38 21 North). It consists of several scattered date palm plantations with genetically different trees. The number of adult date palm plants is estimated to be around 200 000 individuals (Ortos and Lopez-Jimens 2003) and the total area of the palm grove is about 400 hectars (Ferry and Greiner 1997). The average monthly temperature is ranging from 11.2 to 26.6°C and the mean annual rainfall is approximately, 300 mm.

Soil sampling

Twenty three soil samples were collected from several date palm plantations at Elx during May–June 2004. Approximately 500 g of soil were removed with a sterile trowl from a depth of 2–10 cm at each site after first removal of the upper 0–2 cm of top soil. Soil samples were stored in polythene bags at 5°C in the dark and were processed within 1–2 weeks after collection.

Fungal isolation

Five isolation methods, well established for studies on soil fungi, were applied, standard soil dilution plate (Johnsson *et al.* 1959), direct plating method (Warcup 1960), soil treatment with 5% acetic acid (Furuya and Naito 1979), treatment with 70% alcohol (Warcup and Baker 1960), and treatment with 2% phenol (Furuya and Naito 1980). For fungal count, the standard dilution plate technique with 10^{-3} dilution (Johnsson *et al.* 1959) was adopted. The number of colonies of each species/g oven dry soil was calculated. Three types of growth media were used for isolation of the pathogens viz. potato carrot agar (PCA) (20 g peeled potatoes, 20 g carrot, 20 g agar, IL distilled water),

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malt extract agar (MEA. Scharlau, Barcelona, Spain) and potato dextrose agar (PDA, Oxoid, England). Each medium was supplemented with 50 ug.ml chloramphenicol (Sigma, U.S.A) to suppress bacterial growth. Plates for all methods and media (six replicate each) were incubated at 25ºC in the dark. Single colonies were picked from the plates under a dissecting microscope and transferred to appropriate media to allow fungus development. Cultures from single spore were also established. Identification of the two pathogens was based on Ellis (1971, 1976) and Paulin-Mahady *et al*. (2002).

RESULTS AND DISCUSSION

The total fungal counts of the 23 soil samples ranged from $1.1x10^5$ colonies forming units (CFU) to $6x10^5$ CFU per g oven-dried soil. Our fungal count is much higher than those of Al-Doory *et al*. (1959) from, central Iraq ($2.7x10^4$–$2.8x10^5$, CFU/g dried soil) and Abdullah and Zora (1993) from soil at date palm plantations, Southern Iraq (69499–78222 CFU/g dry soil). This lower fungal presence could be due to high salinity and low organic contents in the Iraqi soils.

Figure 1 gives the percent of the total counts for *T. paradoxa* and *T. punctulata* in respect to the total fungal counts in the 23 soil samples/g oven dried soil. Total colony counts for *T. punctulata* ranged between 0.2–3.2% CFU/g dry soil in respect to the total fungal counts, whereas, colony total counts for *T. paradoxa* is ranging between 0.5–4.4% CFU/g dry soil in respect to the total fungal counts.

A notable result from our study was the high frequency of occurrence of *T. paradoxa* (52%) and *T. punctulata* (100%) in soil of date palm plantations at Elx. 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 disease of date palms (Abbas and Abdula 2003; Abbas *et al*. 1997; Djerbi 1983; El-Morsy 1999; Suleman *et al*. 2001).

Unlike the root pathogens, *T. basicola* and *T. thielvioides* were frequently isolated from field soil by using carrot discs and umbelliferous root tissues (McIiveen and Edginton 1972; Yarwood 1946), *T. paradoxa* and *T. punctulata* have been mostly isolated from aerial parts of palms (Abbas and Abdula 2003; Abbas *et al*. 1997; Djerbi 1983; El-Morsy 1999; Suleman *et al*. 2001, 2002). However, the present study reports the frequent isolation of these two pathogens from soil using dilution plate, direct soil plating or by soil treatment either with acetic acid or with phenol (Table 1). Inspite of the occurrence of the disease caused by these two pathogens on date palm in Iraq (Abbas *et al*. 1991, 1997), they have not been isolated from Iraqi soils (Abdullah and Zora 1993; Al-Doory *et al*. 1959).

Both species are characterized by developing thick-walled aleuroconidia, either singly (*T. punctulata*) or in chain (*T. paradoxa*) in addition to the phialoconidia (Figs. 2–5). The thick-walled aleuroconidia are likely to play a role as survival propagules of the two plant pathogens in soils. In conclusion, the widespread existence of such propagules of the two plant pathogens in soil may possibly infect the newly transplanted offshoots of date palms.

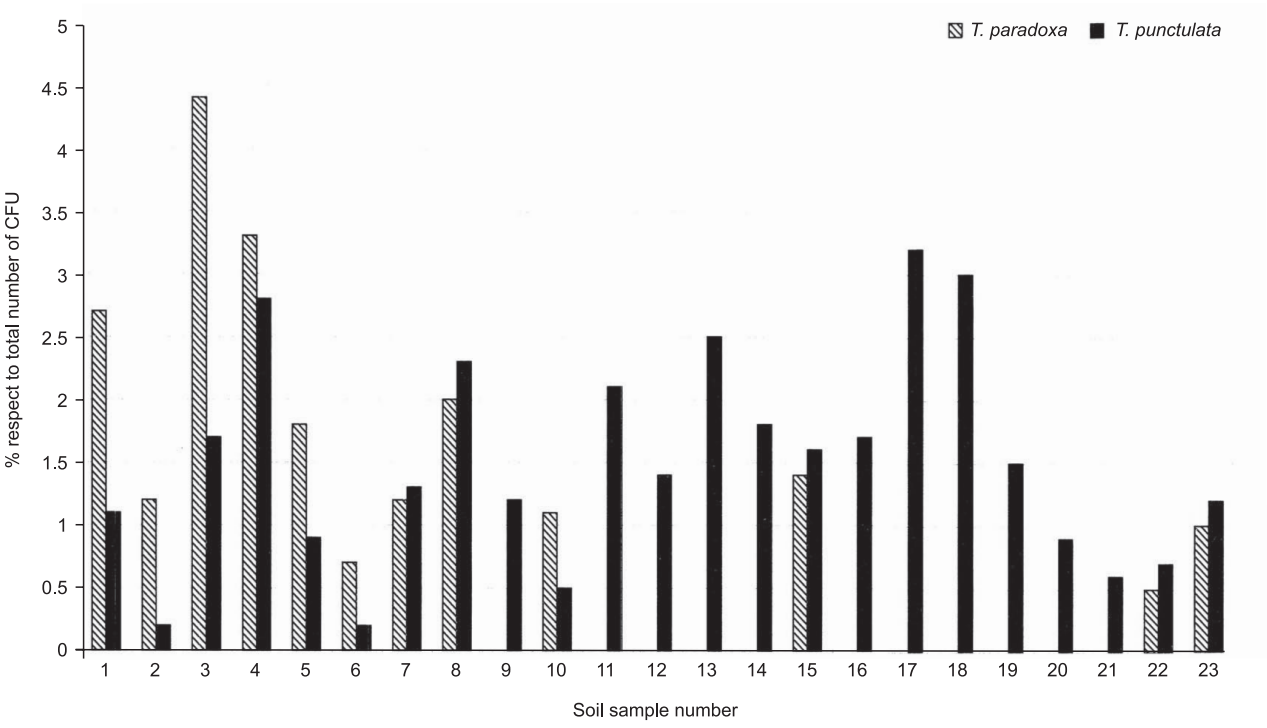
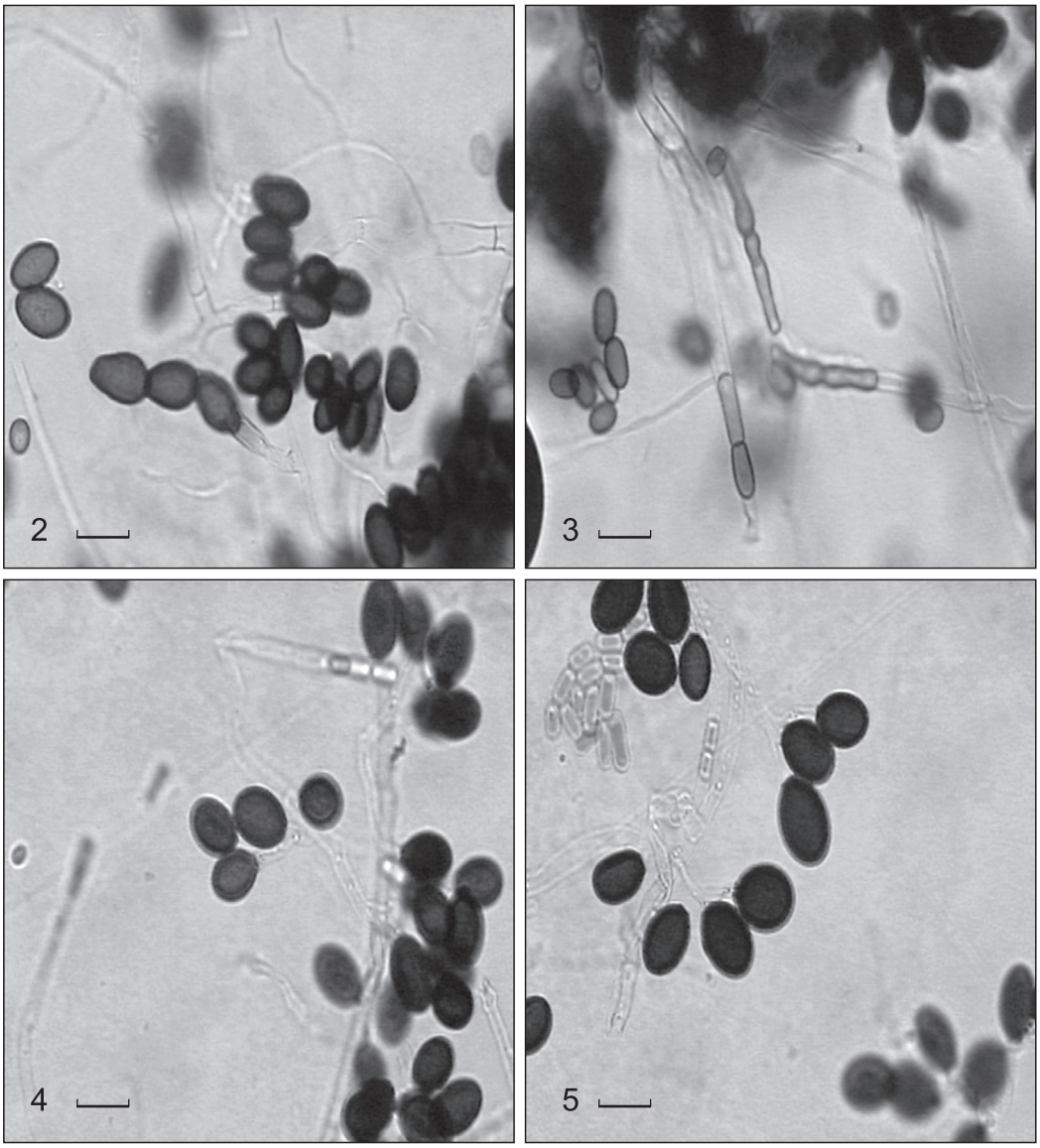


Fig. 1. % total count for *T. paradoxa* and *T. punctulata* in respect to total fungal counts

Table 1. Frequency % of *Thielaviopsis paradoxa* and *T. punctulata* and methods for isolation from soil at date palm plantations at Elx SE Spain

Fungi	1	2
	Frequency %	Method
Thielaviopsis paradoxa	52.17	Ac, Ph, P, D
Theilaviopsis punctulata	100.00	Ac, Ph, P, D

1 – number of positive samples/total number of samples in which the fungi were detected
2 – Ac = Acetic acid treatment, Ph = phenol treatment, P = Direct plating, D = Dilution plating method



Figs. 2–3. *T. paradoxa*: thick-walled aleuroconidia formed in chains and phialoconidia. Scale bar = 10 um

Figs. 4–5. *T. punctulata*: thick-walled aleuroconidia formed singly and phialoconidia. Scale bar = 10 um

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POLISH SUMMARY

WYSTĘPOWANIE DWÓCH PATOGENÓW PALMY DAKTYLOWEJ, *THIELAVIOPSIS PARADOXA T. PUNCTULATA* W GLEBIE Z PLANTACJI PALM DAKTYLOWYCH W ELX, POŁUDNIOWO-WSCHODNIA HISZPANIA

Niniejsza praca dotyczy dwóch patogenów palmy daktylowej – *Thielaviopsis paradoxa* (de Seynes) Hohn i *T. punctulata* (Hennebert) Paulin, Harrington i McNew, pochodzących z gleby plantacji palmy daktylowej w Elx, w południowo-wschodniej Hiszpanii. Patogeny izolowano przy wykorzystaniu metody rozcieńczeń płytkowych, bezpośredniego wykładania na płytki ziemi lub traktowania ziemi kwasem octowym albo fenolem. Obydwa gatunki miały wysoki wskaźnik izolacji. *T. punctulata* był wykryty we wszystkich próbach (100% izolacji), a *T. paradoxa* w 52% izolacji. Ogólna liczba kolonii grzybów wahała się w granicach 1,1 x 10^5–6 x 10^5 jednostek wytwarzających kolonie/1 g suchej ziemi. Z nich, *T. punctulata* stanowił 0,2–3,2%, a *T. paradoxa* 0,5–4,4%. Obydwa gatunki charakteryzowały się wytwarzaniem grubościennych aureokonidiów, pojedynczo (*T. punctulata*) lub w łańcuszkach (*T. paradoxa*), w dodatku do fialokonidiów. Szeroko rozpowszechnione występowanie obydwóch patogenów w ziemi może przyczyniać się do możliwości infekowania nowo przesadzanych sadzonek przybyszowych palm daktylowych.