

## Survey on Leaf Base Rot Disease of Date Palm at New Valley in Egypt

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An emerging and expanding disease of date palm orchards at El-Kharga and El-Dakhla Oases at the New Valley Governorate in Egypt was observed since 2007. The leaves of diseased plants were hanged to the trunk with rotting of the leaf base part. Rotting of leaf base was started partially at one side of the leaf base, then the leaf base was completely rotted causing a weakness leaf which was separated partially and hanged to the trunk. The causal pathogen was isolated from rotted leaf base of date palm and subsequently identified as *Chalara* state of *Ceratocystis radicola* (Bliss) C. Moreau. It is worthy to mention that this is the first time to isolate *Ceratocystis radicola* in New Valley. However, this fungus was recovered from approximately 100% of different locations and scored 50.2% of frequency among all isolated fungi. On the other hand, the other isolated fungi appeared in low frequency.

Seven representative isolates of *C. radicola*, were selected for Inter Simple Sequence Repeat (ISSR) analysis. These isolates represented different morphological variants and different localities in El-Kharga and El-Dakhla Oases, New Valley, Egypt. Based on ISSR marker polymorphisms, results verify that the most distant relationship was scored between the two isolates, Cr.5 from site 5 at El-Kharga Oases and Cr.7 isolated from site 7 at El-Dakhla Oases. The dendrogram of the seven isolates cluster comprised three isolates, Cr. 5 isolated from site 7 at El-Kharga Oases, Cr.6 isolated from site 6 at El-Kharga Oases and Cr. 4 isolated from site 4 at El-Dakhla Oases which were closer in their relationship. The results highlight that attention must be given to the importance of *C. radicola*, since it was isolated from all fields and scored the highest values on the fungal frequency at all locations of El-Dakhla and El-Kharga Oases.

**Keywords:** *Ceratocystis radicola*, date palm, DNA, leaf base rot.

Date palm (*Phoenix dactylifera* L.) is one of the most important fruit trees growing in Egypt, as well as in the Arabian world and some neighbouring countries and represents a good cash crop for many farmers. Palm diseases are among the major factors that affecting the products. Fungi are known as the most causal pathogens on date palm trees, they also are vulnerable to infection with some destructive diseases which are responsible for decline and considerable losses in the number of trees (Elliott and Broschat, 2001; Summerell *et al.*, 2001; Zaid *et al.*, 2002; Smith *et al.*; 2003, Garofalo and McMillan, 2004; Uchida, 2004 and Elliott, 2006). Under local conditions at the New Valley Governorate several investigators demonstrated that the fungus *Chalara* (*Thielaviopsis*) *paradoxa* and some decline diseases attacked date palm trees causing trunk rot, black scorch and leaf base rot.

The dominant fungi associated with rotted leaf base of date palm offshoots were *Fusarium oxysporum*, *Diplodia phoenicum*, *Alternaria* spp., *Pestalotiopsis* sp. and *Rhizoctonia solani* (Rashed, 1998; El-Morsi, 1999; El-Morsi, 2004 and El-Deeb *et al.*, 2007).

Although, *Ceratocystis paradoxa* (Dade) C. Moreau [anamorph: *Thielaviopsis paradoxa* (de Seynes) Hohn.] and *C. radicola* (Bliss) Moreau [anamorph: *T. punctulata* (Hennebert) Paulin, Harrington, et McNew, *comb. nov.*] are two pathogens commonly found either alone or in combination associated with several disease symptoms on palm trees. However, these fungi can infect any part of the palm tree, and symptoms are often expressed as black scorched leaves, trunk rot, neck bending or inflorescence blight (Abbas, *et al.*, 1997; Djerbi, 1983; Suleman *et al.*, 2001; Zaid *et al.*, 2002; Abbas and Abdulla, 2003 and El-Gariani *et al.*, 2007). Molecular markers such as inter simple sequence repeat (ISSR) markers have been successfully used as tools for understanding the phylogenetic relationships of fungi (Chadha and Gopalakrishna, 2007 and Schwarzenbach *et al.*, 2007). ISSR is a simple technique, requires no sequence information and is carried out using a single primer based on a simple repeat. Using ISSR marker polymorphisms, insures the relation among the fungi isolated from different locations under study and may also answer the question how the pathogen can move from field to other or from an Oasis to another in the New Valley. The aim of this study was to realize an emerging and expanding disease of date palm at El-Kharga and El-Dakhla Oases at the New Valley Governorate which was observed since 2007.

## Materials and Methods

### 1. Isolation of the pathogen:

Samples from naturally infected date palm trees showing typical symptoms of the leave base rot (Fig.1) were collected from seven orchards, each one about 5 to 10 hectares at different sites in El-Kharga and El-Dakhla Oases, New Valley, Egypt. The base part of the diseased leaf was cut into small pieces, rinsed several times in sterilized distilled water, disinfected by 0.1% sodium hypochlorite solution for one minute, followed by washing in three changes of sterilized water and dried between folds of sterilized filter paper. The sterilized plant fragments were aseptically transferred to Petri dishes containing 10 ml of PDA medium and incubated at 25°C for 5 days.

### 2. Identification of the isolated fungi:

Identification of *C. radicola* isolates were carried in the Plant Pathology Unit of Desert Research Centre (DRC) according to Barnett and Hunter (1972) and Booth (1971). Identification of fungi was confirmed in the Mycological Centre at the Faculty of Science, Assiut University, according to Moubasher (1993).

Frequency of the isolated fungi from different locations was calculated according to the following equation:

$$\text{Frequency (\%)} = \frac{\text{Number of isolates for each species}}{\text{Total number of isolates}} \times 100$$

### 3 - DNA extraction

Seven representative isolates of *C. radiculicola* were selected to use for ISSR analysis. These isolates represented different morphological variants and different localities in El-Kharga and El-Dakhla Oases, New Valley (Table 1). These isolates were grown on PD broth at room temperature for 10 days. DNA extractions were performed using the protocol of DeScenzo and Harrington (1994).

**Table 1. Source of *C. radiculicola* isolates used for DNA analysis**

Isolate No.	Oasis	Orchards	Sites
Cr.1	El-Kharga	Ain Amer (a)	(site 1)
Cr.2	El-Kharga	El Malah (a)	(site 2)
Cr.3	El-Kharga	Ain Sokhara	(site 3)
Cr.4	El-Dakhla	Sala-Mot	(site 4)
Cr.5	El-Kharga	Ain Amer (b)	( site 5)
Cr.6	El-Kharga	El Malah (b)	( site 6)
Cr.7	El-Dakhla	El Mahsra	(site 7)

### 4- DNA sequencing

Genomic DNA from each site was isolated according to the method of Junhans and Metzlatt (1990).

### 5- ISSR-PCR analysis

ISSR-PCR reactions were conducted according to Sharma *et al.* (1995) using seven preselected primers (Table 2) which were synthesized by (Metabion Germany) for seven *C. radiculicola* isolates (Table 1). The reaction conditions were optimized and mixtures were prepared (25 µl total volumes) consisting of the following: 1.0 µl dNTPs (10 mM), 1 µl Taq DNA. polymerase (1U/1µl), 2.5 µl 10 X buffer, 3 µl MgCl<sub>2</sub> (15 mM), 1.0 µl Primer (10mM), 1.0 µl Template DNA (50 ng/µl) and 15.5 µl H<sub>2</sub>O up to 25 µl. Amplification was carried out in Stratgene Robocycler Gradient 96 which was programmed for 45 cycles as follows: Denaturation (one cycle) at 94°C for 2 minutes, followed by 30 cycles: as follows 94°C for 40 second, 44°C for 45 sec, 72°C for 1 minute and 30 sec, and finally one cycle extension at 72°C for 20 minutes, and 4°C (infinite)

**Table 2. List of ISSR primers and their nucleotide sequence**

Primer No.	Sequence
HB1	(CAA) 5
HB2	(CAG) 5
17898A	(CA) 6 AC
17898B	(CA) 6 GT
17899A	(CA) 6 AG
17899B	(CA) 6 GG
HB15	(GTG) 3 GC

Agarose gel electrophoresis (1.2%) ultra pure (GIBCOBRL) was used for resolving the PCR amplification products according to Sambrook *et al.* (1989). The run was performed for one hour at 120 volt in Biometra submarine (40x20 cm). Bands were detected on UV- transilluminator and photographed by Biometra Bio Doc Analyze 2005.

A marker one Kb plus DNA 100 bp Ladder 1µg /µl (ferments) was used with molecular sizes of: 100, 200, 300, 400, 500, 600, 700, 800, 900, 1031, 1200, 1500, 2000, 2500, 3000, 3500, 4000, 5000, 6000, 8000 and 10000 bp.

## Results

### Symptoms:

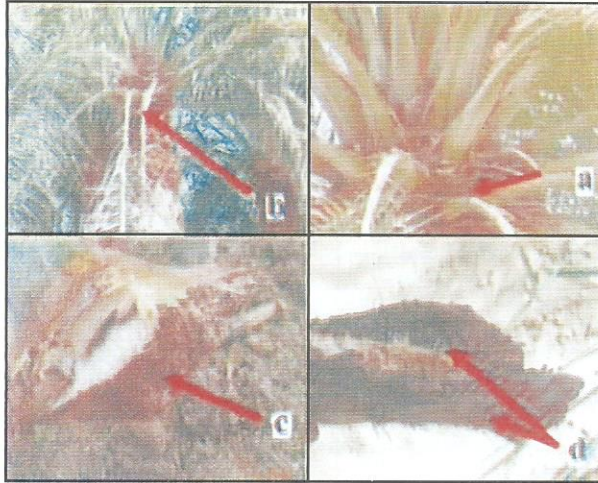
An emerging and expanding disease of date palm grown in El-Kharga and El-Dakhla Oases at the New Valley Governorate was observed since 2007. The leaves of the diseased date palm trees were hanged to the trunk associated with rotting of leaf base part. Rotting of leaf base was established partially at one side of the leaf base, then the leaf base was completely rotted causing a weakness on the leaf which was separated partially and hanged to the trunk. At the last stage of infection, the leaves showed the wilt symptoms (Fig. 1).

### Frequency of the isolated fungi from different orchids at El-Kharga and El-Dakhla Oases:

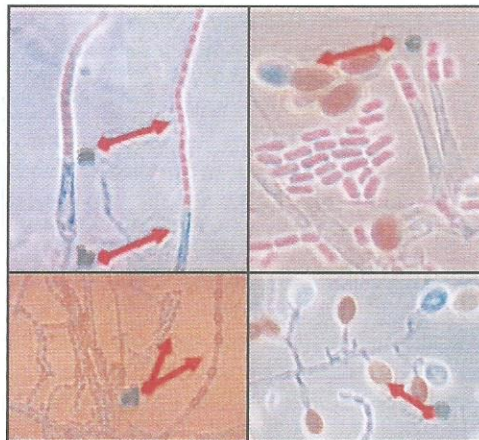
The main and most prevalent fungus isolated from diseased palms was identified as *Chalara* state of *Ceratocystis radicola* (Bliss) C. Moreau (Fig. 2). *C. radicola* was recovered approximately from 100% of different sites at El-Kharga and El-Dakhla Oases and scored 51.2% followed by *Fusarium* sp., being 14.6% frequency (Table 3).

At El-Kharga Oases, *C. radicola* was the most common fungus, recorded 48.7, 39.5 and 56.2% at Ain Sokhara (site 3), El Malah (site 2 and 6) and Ain Amer (site1 and 5), respectively. At El-Dakhla Oases, *C. radicola* was the most prevalent and scored 62.9 and 48.8% at El Mahsra (site 7) and Sala -Mot (site 4), respectively. On the other hand, the frequency (%) of the other isolated fungi showed low values ranged between 0.3% for *Ulocladium* sp. and 5.9% for *Alternaria* sp. (Table. 3). Data in Table (3) also show that *Trichoderma* sp. was only isolated from Ain Amer (site1 and 5), *Ulocladium* sp. from El Malah (site 2 and 6) and *Aurobasidium* sp. from Ain Sokhara (site 3) at El-Kharga Oases.

The results suggested that attention must be given to the importance of *C. radicola*, since it was isolated from all orchids and scored the highest values of frequency at all locations of El-Dakhla and El-Kharga Oases.



**Fig .1. Symptoms on date palm leaves hanged to the trunk of the diseased trees located in El-Kharga and El-Dakhla Oases at the New Valley**  
**a- Rotting of leaf base established partially at one side of the leaf base**  
**b- Leaves of date palm hanged to the trunk (attached to the trunk).**  
**c- The base part appears rotted with dark fibrous.**  
**d- The leaf base is completely rotted.**



**Fig .2. Chalara state of *Ceratocystis radicicola* (Bliss) C. Moreau**  
**a- Conidial chain.**  
**b- Phialides.**  
**c- Chlamydo spores.**  
**d- Young and mature phialoconidia.**  
**e- Solitary chlamydo spores.**

**Table 3.** The frequency percentage of fungi isolated from rotted leaf base part of date palm at El-Kharga and El-Dakhla Oases in New Valley

Fungus	Frequency (%) of isolated fungi					Ave.
	El-Kharga			El-Dakhla		
	Ain Sokhara (site 3)	El Malah (site 2 and 6)	Ain Amer (site 1 and 5)	El Mahsra (site 7)	Sala-mot (site 4)	
<i>Alternaria</i> sp.	7.3	7.8	0.0	0.0	14.6	5.9
<i>Aurobasidium</i> sp.	7.3	0.0	0.0	0.0	0.0	1.5
<i>Bipolaris</i> sp.	0.0	6.8	0.0	5.7	9.8	4.4
<i>Ceratocystis radicola</i>	48.7	39.5	56.2	62.9	48.8	51.2
<i>Cladosporium</i> sp.	0.0	1.4	7.4	0.0	0.0	1.7
<i>Chrysonilia</i> sp.	4.9	0.0	0.0	0.0	0.0	1.0
<i>Curvularia</i> sp.	0.0	6.7	0.0	0.0	4.9	2.3
<i>Diplodia</i> sp.	4.9	6.5	2.9	14.3	0.0	5.7
<i>Drechslera</i> sp.	0.0	4.1	0.0	0.0	0.0	0.8
<i>Fusarium</i> sp.	12.2	14.3	15.6	11.4	19.5	14.6
<i>Helminthosporium</i> sp.	7.3	0.0	0.0	0.0	2.4	1.9
<i>Macrophomina phaseolina</i>	4.9	0.0	0.0	0.0	0.0	1.0
<i>Pestalotiopsis</i> sp.	0.0	11.5	9.5	0.0	0.0	4.2
<i>Trichoderma</i> sp.	0.0	0.0	8.3	0.0	0.0	1.7
<i>Verticillium</i> sp.	2.4	0.0	0.0	5.7	0.0	1.6
<i>Ulocladium</i> sp.	0.0	1.4	0.0	0.0	0.0	0.3

*Characterization of C. radicola based on ISSR analysis:*

For ISSR analysis, DNAs of the seven isolates of *C. radicola* were subjected to PCR -ISSR against seven primers (HB01, HB02, 17898 A, 17898 B, 17899 A, 17899 B and HB15) as shown in Table (4) and Fig. (3). A total of 68 amplicons (amplified fragments) were generated by the seven primers in which 57 of them were polymorphic (83.82%). The number of amplicons per primer varied from eight (HB15) to twelve (17898 A). The higher number (5) of monomorphic amplicons was scored for 17898 A primer (Table 4).

Based on ISSR marker polymorphisms, similarity matrix was developed by SPSS computer package (Table 5). The analysis was based on the number of markers that showed differences between any given pair of isolates. The closest relationship was scored between the two isolates, Cr.5 isolated from (site 5) and Cr.6 (site 6) at El-Kharga Oases followed by the two isolates Cr.5 and Cr.4 (site 4) of El-Dakhla Oases and between Cr.5 and Cr.3 (site 3) at El-Kharga Oases recorded similarity of 0.885, 0.876 and 0.817, respectively. On the other hand, the most distant relationship was scored between the two isolates, Cr.5 (site 5) at El-Kharga Oases and Cr.7 (site 7) at El-Dakhla Oases. The dendrogram (Fig. 4) of the seven isolates cluster was comprised three isolates, Cr.5 isolated from site 7 at El-Kharga Oases, Cr.6 isolated from site 6 at El-Kharga Oases and Cr.4 isolated from site 4 at El-Dakhla Oases, that were more closest in their relationship.

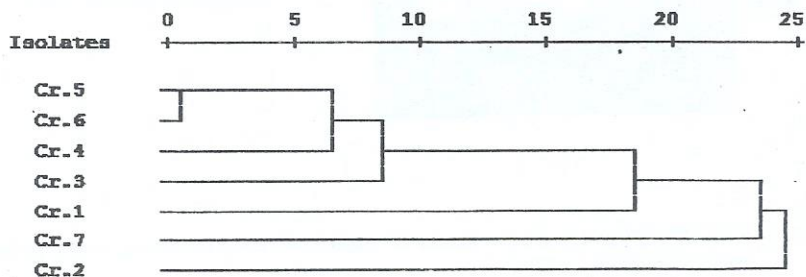
**Table 4. Amplification results of the seven ISSR primers for seven *C. radiculicola* isolates**

Primer code	TAF	PB	P%	Genotype*														
				Cr.1		Cr.2		Cr.3		Cr.4		Cr.5		Cr.6		Cr.7		TSM
				AF	SM	AF	SM	AF	SM	AF	SM	AF	SM	AF	SM	AF	SM	
HB01	10	9	90.00	6	0	5	0	6	0	6	0	6	0	5	0	7	1	1
HB02	11	8	72.70	8	1	7	0	7	0	7	0	5	0	5	0	5	0	1
17898 A	12	11	91.67	8	3	6	1	7	0	7	0	7	0	7	0	7	1	5
17898 B	9	8	88.89	6	0	7	1	4	0	2	0	4	0	4	0	4	1	2
17899 A	9	8	88.89	5	0	2	1	7	1	3	0	4	0	5	0	5	1	3
17899 B	9	7	77.78	7	2	5	0	5	0	5	0	4	0	3	1	6	0	3
HB15	8	6	75.00	8	0	7	0	4	1	4	0	5	0	5	0	6	1	2
Total	68	57	83.82	48	6	39	3	40	2	34	0	35	0	34	1	40	5	17

\* TAF= Total number of amplified fragments, PB= Polymorphic bands, P%= polymorphism percentage, AF= Amplified fragments/genotype, SM= Genotype- specific marker including either the presence or absence of a given band, TSM= Total number of specific markers.

**Table 5. Similarity matrices among the seven *C. radiculicola* isolates using seven primers based on ISSR analysis**

<i>C. radiculicola</i> isolates	Cr.1	Cr.2	Cr.3	Cr.4	Cr.5	Cr.6
Cr.2	0.706					
Cr.3	0.764	0.796				
Cr.4	0.807	0.729	0.807			
Cr.5	0.752	0.764	0.817	0.876		
Cr.6	0.705	0.786	0.816	0.815	0.885	
Cr.7	0.717	0.705	0.692	0.764	0.703	0.752



**Fig. 3. ISSR fingerprints of the seven *C. radiculicola* isolates using seven primers.**

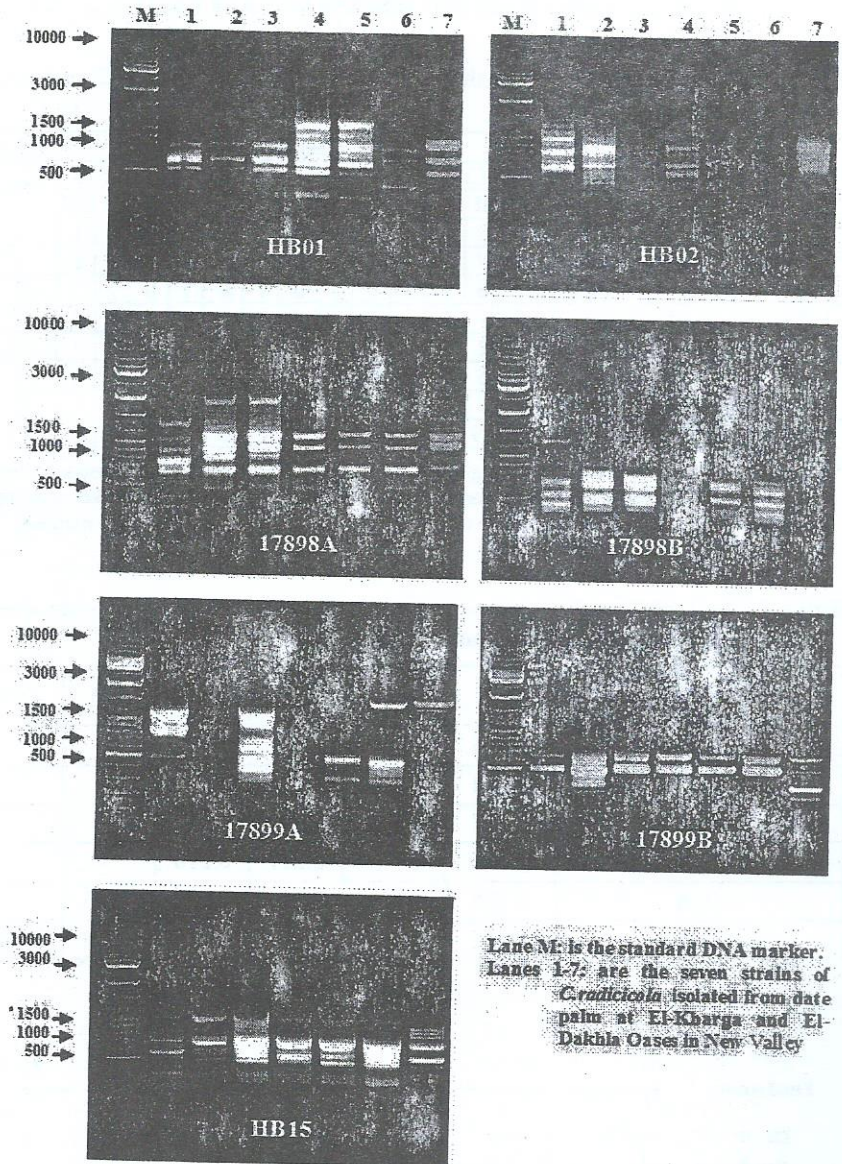


Fig. 4. The Dendrogram of the seven *C. radicata* isolates using seven primers based on ISSR analysis.



### Discussion

In the present study, the results showed that *Ceratocystis radicola* (Bliss) C. Moreau was the most prevalent fungus isolated from diseased palms with 100% recovering from different sites at El-Kharga and El-Dakhla Oases, followed by *Fusarium* sp. Similar results were reported by Besri (1982) and Djerbi (1983). In addition, previous studies on date palm diseases under local conditions at New Valley reported that the fungus *Chalara* (*Thielaviopsis*) *paradoxa* and some decline diseases attacked date trees causing trunk rot, black scorch and leaf base rot (Rashed, 1998, El-Morsi, 1999 and El-Morsi, 2004).

In line with the previous studies, the authors found that *C. radicola* isolated from El-Kharga and El-Dakhla Oases was the most prevalent one and scored the highest values of frequency at New Valley Oases. While, the other isolated fungi had a lower values of frequency at different orchids. These results are in agreement with those obtained by Abbas and Abdulla (2003), El-Deeb *et al.* (2007) and El-Gariani *et al.* (2007). Results of the present investigation emphasize that attention must be given to the importance of *C. radicola*, since it was isolated from all fields and scored the highest values of frequency at all locations of El-Dakhla and El-Kharga Oases. This may be due to the ability of *C. radicola* to infect any part of the palm tree (Djerbi, 1983; Abbas *et al.*, 1997; Suleman *et al.*, 2001; Zaid *et al.*, 2001; Abbas and Abdulla, 2003; El-Deeb *et al.*, 2007 and El-Gariani *et al.*, 2007). However, symptoms are often expressed as black scorched leaves, trunk rot, neck bending or inflorescence blight. Moreover, most infections occur in non-lignified or lightly lignified tissue. The fungus often produces volatile substances, specifically ethyl acetate and ethyl alcohol, which make the odour of the diseased tissue as a fermented fruit odour (Garofalo and McMillan, 2004 and Elliott, 2006).

Based on ISSR marker polymorphisms, the results show that the most distant relationship was scored between the two isolates, Cr.5 from site 5 at El-Kharga Oases and Cr.7 isolated from site 7 at El-Dakhla Oases. The dendrogram of the seven isolates cluster comprised three isolates; Cr.5 isolated from site 7 at El-Kharga Oases, Cr.6 isolated from site 6 at El-Kharga Oases and Cr.4 isolated from site 4 at El-Dakhla Oases and were closer in their relationships. These relations between different isolates may be due to that infection can occur in one field and move over all fields. The fresh wounds in the different palm tree organs predisposed the most suitable conditions to infection. Wounds can occur naturally, such as trunk cracks due to excess water uptake. Also insects (such as Ambrosia beetles), birds (sapsuckers pounding on the trunk), rats, and other mammals can cause wounds. Blowing solid objects during a wind storm can strike the trunk and cause a fresh wound (Elliott, 2006).

In additions, wounds can occur with nails and climbing spikes, or during the digging and transplanting process and during the trimming process with the careless use of the pruning tool. Pulling a leaf off the trunk, when the leaf petiole still has green tissues can create a fresh wound. The pathogen can also spread from one palm to another by wind and water as well as insects or rodents. Chlamydo spores can survive in the soil for long periods and can infect via contaminated soil (Harrington, 1988 and Elliott, 2006).

### Conclusion

The present study is paying attention for an emerging and expanding disease of date palm orchids at El-Kharga and El-Dakhla Oases at the New Valley Governorate in Egypt which was observed since 2007. The fungal pathogen was isolated from rotted with leaf base of date palm and subsequently identified as *Chalara* state of *Ceratocystis radicicola* (Bliss) C. Moreau. Further studies are needed for proving the pathogenicity of the isolated fungi and their control.

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(Received 04/07/2010;  
in revised form 19/09/2010)

## حصرمض تعفن قواعد جريد نخيل التمر

بالوادي الجديد - مصر

ثناء عبد العزيز مرعي وخالد إسماعيل زكي

قسم وقاية النبات - مركز بحوث الصحراء- المطرية - القاهرة.

لوحظ منذ عام ٢٠٠٧ ظهور وانتشار مرض في أشجار نخيل التمر في الواحات الخارجية والداخلة بالوادي الجديد - مصر، حيث ظهر جريد النخيل المصاب منهذلاً. ويفحص الأشجار المصابة في عدد ٧ حقول مصابة لوحظ تعفن في قاعدة الجريدة المتصل بالجذع حيث يبدأ العفن في احد الجوانب ثم تتعفن قاعدة الجريدة كلها مسببة ضعف الجريدة وتهدلها ناحية جذع النخلة ثم تذبل وتموت.

تم عزل الفطر المسبب من قواعد جريد النخل المصاب وتم تعريفه على أنه

*Chalara state of Ceratocystis radicicola* (Bliss) C. Moreau

والجدير بالذكر أنه تم عزله من جميع قواعد الأوراق المصابة من جميع المواقع بنسبة ١٠٠% وقد سجل نسبة ٥٠،٢% من متوسط نسبة تكرار الفطريات المعزولة معه والتي ظهرت بنسب تكرارية قليلة في جميع الحقول المصابة بالواحات الداخلة والخارجة مع العلم بأن هذا أول تسجيل لهذا الفطر في الوادي الجديد.

تم اختيار ٧ عزلات من الفطر *Ceratocystis radicicola* مختلفة مورفولوجيا وتمثل مختلف مواقع الدراسة في الواحات الداخلة والخارجة لاختبارها وتوصيفها وتحديد درجة القرابة بينها باستخدام *Inter Simple Sequence Repeat (ISSR)* وبالتالي معرفة سبب انتشار الإصابة وانتقالها من حقل لأخر داخل الواحات موضع الدراسة.

وبناء على اختبار المعلمات الوراثية أوضحت النتائج وجود علاقة قوية بين العزلة رقم ٥ والمعزولة من موقع رقم ٥ بالواحات الخارجية والعزلة رقم ٧ المعزولة من موقع رقم ٧ بالواحات الداخلة. كما اظهر تحليل شجرة القرابة ان العزلات ٦٥ و ٧ المعزولة من مواقع ٦ و ٧ بالواحات الخارجية وموقع ٤ بالواحات الداخلة على التوالي كانت أكثر العزلات في درجة القرابة. ولذلك فإن نتائج البحث تلقى الضوء على أهمية هذا الفطر، بما أنه قد تم عزله من جميع الحقول المصابة وكان أكثر الفطريات المعزولة في نسبة التكرار في الواحات الخارجية والداخلة بالوادي الجديد. وكذلك فإن درجة القرابة العالية بين السلالات تشير الى إمكانية انتشار الفطر عبر الواحات بالطرق المختلفة وإثناء عمليات الخدمة.