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New Record of the Pyrenomycete *Coniochaeta velutina* Causing Leaf Blight of Date Palm

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Abstract. *Coniochaeta velutina (*Fuckel) Munk was isolated for the first time from blighted leaves of date palm trees grown in Al-Qassim region, Saudi Arabia. Blighted leaflets developed a golden-yellow to rusty color with very small (pin-head-like) dark ascomata scattered on both surfaces of infected leaflets. Koch's postulates were applied using four months old date palm seedlings grown *in vitro* as well as leaves of five years old mature trees. Histological studies of infected tissues revealed that the fungus grew both intra-and intercellularly in the parenchymatous cells of the cortical region. Hyphal growth, however; could also be detected in the xylem vessels of artificially inoculated seedlings. This is the first record of *C. velutina* as a pathogen of date palm.

Introduction

There are several reports on the occurrence of various leaf spot diseases of date palm in Saudi Arabia. Symptoms of these diseases differ greatly based mainly on the organism(s) involved. For instance, Graphiola leaf spot caused by *Graphiola phoenicis* was found prevailing in the Eastern Province of Saudi Arabia [1, p. 111]. Signs of this disease are found on both surfaces of the pinnae as rounded yellowish pustules elevated about 2 mm from the leaf surface. Another disease, brown leaf spot of date palm was found in the Eastern and the Central provinces. This is a complex disease caused by *Alternaria alternata* and *Xylohypha nigrescens* [2]. In this case the disease is characterized by minute scattered dark brown spots surrounded with yellow necrotic areas on both surfaces of the pinnae. El-Meleigi *et al*. [3] surveyed foliar fungal diseases of date palm in Al-Qassim region (Central Saudi Arabia) and reported that *Alternaria alternata* and *Drechslera biseptata* were the most common pathogens found associated with date palm leaf spots in the region. *D. biseptata* was associated with elongated spots that are characterized by grayish color with definite dark edges. *A. alternata*, however; caused irregular dark brown to black spots. Later, and in the same region, Al-Rokibah

[4] reported the occurrence of a leaf blight disease of date palm caused by *Glomerella cingulata*. The symptoms of this disease appeared as reddish brown spots with distinct dark edges.

During routine investigations of date palm orchards in Al-Qassim region, a new disease was observed that was causing complete blight of many leaves of the affected trees. The disease was of sporadic distribution but infested trees were severely affected. The aim of this study was to identify the fungus found associated with this disease and to determine its pathogenicity on date palm.

Materials and Methods

Isolation and identification of the etiological agent

Date palm leaves with blight symptoms were used for the isolation of the causal agent. Leaflets (pinnae) tissues were cut into 1 cm^2 squares, surface sterilized in 0.5% sodium hypochlorite for 1 min, then tissues were rinsed in sterile distilled water (SDW) for another minute. After air drying on sterile filter paper, tissues pieces were placed on acidified potato dextrose agar (APDA) medium (pH=4). Plates were incubated at 25° C for about two weeks. After incubation, the developed fungal growth was purified using the hyphal tip technique, and transferred to corn meal agar (CMA) to induce sporulation. The developed fungal growth on APDA and CMA was identified depending on morphological and cultural characteristics, Ainsworth et al. [5, p. 109] and Taylor [6]. Acidified potato dextrose agar medium was compared with three other agar media commonly used to culture and enhance sporulation of fungi. These culture media were Czapek Dox agar (CDA), corn meal agar (CMA), and carrot agar (CA). Linear growth of the isolated fungus was measured after 2 weeks on the above media. Sporulation of the fungus under investigation was also studied on the above mentioned culture media. This experiment was repeated twice and data were combined. Means were compared using Duncan's multiple range test.

Pathogenicity tests

Two pathogenicity tests were conducted using the fungus under investigation. In the first inoculation experiment, four months old date palm seedlings cv. Ruzeiz were grown in vitro on Murashige and Skoog (MS) medium as described by Al-Wasel and Warrag [7]. The second pathogenicity test was conducted according to the method described by Sheir *et al*. [2] using five years old date palm trees cv. Ruzeiz. Fungal cultures were grown on CMA medium at 25°C . The inoculum was prepared by flooding the agar surface with 10 ml of SDW, then the surface was scraped with a sterile spatula. The resulting spore suspension was filtered through 4 layers of cheesecloth, the ascospores concentration was adjusted to $1x10^7$ spores/ml using a hemacytometer. In the first experiment, date palm seedlings were injected in the crown area with 2 ml of the above mentioned spore suspension using a sterile hypodermic needle and syringe.

Control seedlings were injected only with SDW. As for the second pathogenicity test, the epicuticular wax layer was abraded from the surface of the leaflets as recommended by Moustafa *et al*. [8]. The spore suspension was then sprayed onto the leaflets untill run-off. Control leaflets were sprayed with SDW only. Inoculated portions of the leaves were then covered with black plastic bags for 48-hr to maintain high humidity.

Histological studies of inoculated date palm seedlings

Artificially inoculated seedlings of date palm cv. Ruzeiz were fixed in a solution of formalin, acetic acid and ethyl alcohol 50% (5: 5: 90 %) for 48 hr., washed in ethyl alcohol 50% , then dehydrated by passage through a series of ethyl alcohol, cleaned in xylol, and embedded in paraffin wax [9].Serial microtome sections (10-15µ) were made and adhered to microscope slides using Haupt's gelatin adhesive mixed with formalin . The stain combination used consisted of 15 aqueous solution safranin O, plus picroaniline blue (25 ml saturated aqueous solution of aniline blue in 100 ml saturated aqueous solution of picric acid) as described in Cartwright's method [10].

Results

Disease symptoms

Symptoms started from the top of the leaf and moved downward to the leaf base. The green color started fading from the top of affected leaflets and moved down. Later, the profuse sporulation (conidial) of the fungus on infested leaflets gave these leaflets the characteristic golden-yellow to rusty color. Later, small black ascomata were found scattered on both surfaces of the leaflets. Artificial inoculation of mature tree leaves, resulted in the appearance of the same symptoms described above (Fig. 1, b). However, inoculation of young seedlings (four months old) lead to complete blight of infected seedlings. The color of the seedlings faded, turned light brown then assumed a dark brown color with several black perithecia scattered on the tissues of the whole seedling including the root (Fig. 1, c). No disease symptoms were observed on control plants of both inoculation experiments.

Isolation and identification of the etiological agent

One organism was consistently isolated from leaf tissues showing the golden-yellow to rusty color blight symptoms. Growth on APDA was slow, with 2.4 mm radial growth after two weeks (Table 1). Colonies appeared white at first, then it turned dark gray. Conidiophores were hardly distinguishable from hyphal tips. Conidia were produced in abundance on infected tissues as well as on culture media. Conidia were hyaline, single-celled, ovoid, smooth, 4-6 x 1.5-3 µ. Numerous secondary conidia were formed by budding. Perithecia developed on APDA but only after about 4-5 weeks. That perfect stage, however; developed more readily on CMA and CA than on APDA or CDA (Table 1). After fourty days on APDA, perithecia were globose-ovoid,

black,gregarious with setae (15-40 x 3-4 µ), the perithecial diameter is up to 450 µ. Ascospores dark brown, ovoid, 7-9 x 5-7 µ, occasionally with elongate germ slit. Concering all morphological characteristics, the fungus under investigation was identified according to Ainsworth *et al*. [5], Taylor [6] and Cooke *et al*. [11] as *Coniochaeta velutina* (Fuckel) Munk .

Table 1. Sporulation and colony diameter of *Coniochaeta velutina* after two weeks of growth on acidified potato dextrose (APDA), Czapek Dox agar (CDA), corn meal agar (CMA) and carrot agar (CA)

Media	Perithecial formation	Colony diameter (cm)
APDA	-[x]	2.4 a[y]
CDA	-	2.7 a
CMA	++	2.3 a
CA	+	2.1 a

[x] Perithecial formation: -= no perithecia formed, += limited number of perithecia formed, ++= large number of perithecia formed.
[y]Means followed by the same letter are not significantly different (P=0.05) according to Duncan's multiple range test.

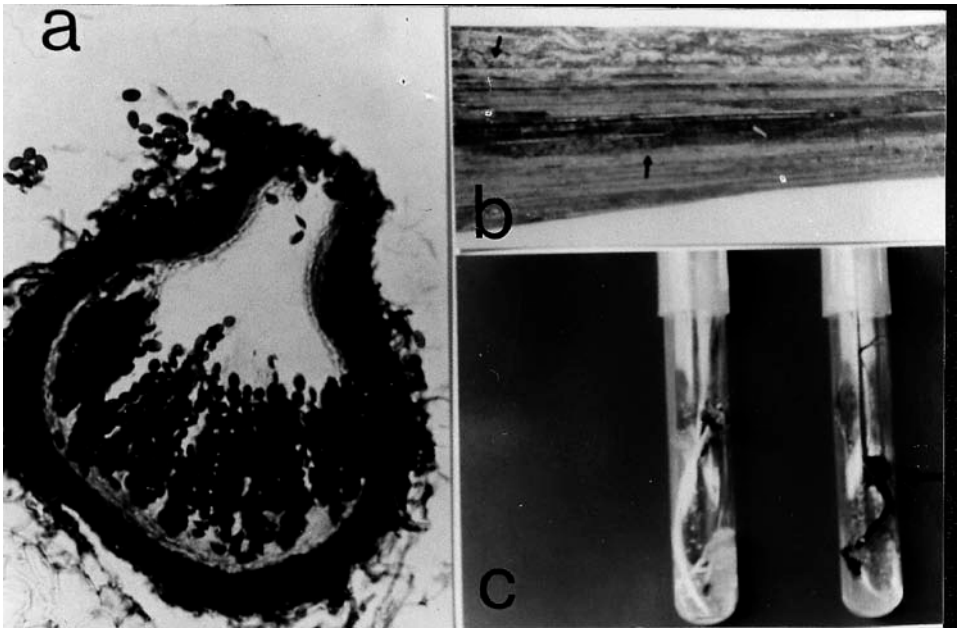

Fig.1. a) Enlarged perithecia (x 600) of the fungus *Coniochaeta velutina* developed on the surface of the plumule base; (b) Blighted leaflet of date palm assuming the golden-yellow to rusty color, the small arrow points to the very small dark perithecia; (c) Infected date palm seedling (right) showing the complete blight symptoms as compared with check (left).

Pathogenicity tests

Leaf blight symptoms similar to those found in the field were observed only in case of the second inoculation technique. Inoculated leaflets developed golden-yellow to rusty color one week after infestation. Perithecia started developing on inoculated leaflets about 10 days after inoculation. On the other hand, inoculated young seedlings grown *in vitro* on MS medium, began loosing its green color starting from the apex and extending towards the base only three days after inoculation. After one week the whole seedling became dark brown (Fig.1-c) with black perithecia scattered on its tissues including the plumule, cotyledonary sheeth and the root.

Histological studies of date palm seedlings infected with *C. velutina*

Transverse sections in roots and plumule bases of seedlings infected with *C. velutina* revealed the systemic nature of infection. Inter and intracellular invasion of the parenchymatous cells of the cortical region by fungal mycelia was evident in root and plumule base transverse sections (Figs.2 and 3). The heavy establishment of the fungal mycelium in the ground tissue of the root led to complete degeneration of many of the parenchyma cells, the fungus (stained blue) could also be detected in the xylem vessels (Fig.3). A hyphal network was formed on many parts of the surface of artificially inoculated seedlings. Dense agglomerations of hyphae were formed and perithecia were developed from these agglomerations (Figs.2 and 3).

Discussion

The morphological characteristics of the Pyrenomycete described in this study conformed to those previously illustrated for *C. velutina* [5, 6, 11]. A representative culture of this fungus was delivered to the International Mycological Institute (now known as CABI Bioscience, UK), where the fungus was identified and designated as: IMI 371201. This species has primarily been isolated from forest trees in temperate environments such as Canada and England [12,13]. This is the first record of this fungus as a pathogen of date palm and also first record for its existence in the Near East. Basham et al. [12] reported that *C. velutina* had always been associated with stem injury of sugar maple trees. They suggested that *C. velutina* may be an important wound pathogen. Results presented in this study were in agreement with the previous statement. In the two inoculation experiments conducted in this study, injury of date palm tissues was required before successful inoculation occurred. In the open field, injury may easily take place on date palm leaves. Desert storms carrying sand particles may present a good example. The pathogenicity tests conducted in this study revealed that young date palm seedlings were more susceptible to infection with *C. velutina* than were mature trees. This phenomenon has been reported before for other diseases. For instance, young cotton and Japanese holly seedlings [14,15] were found to be more susceptible to infection with *Thielaviopsis basicola* than were mature plants.

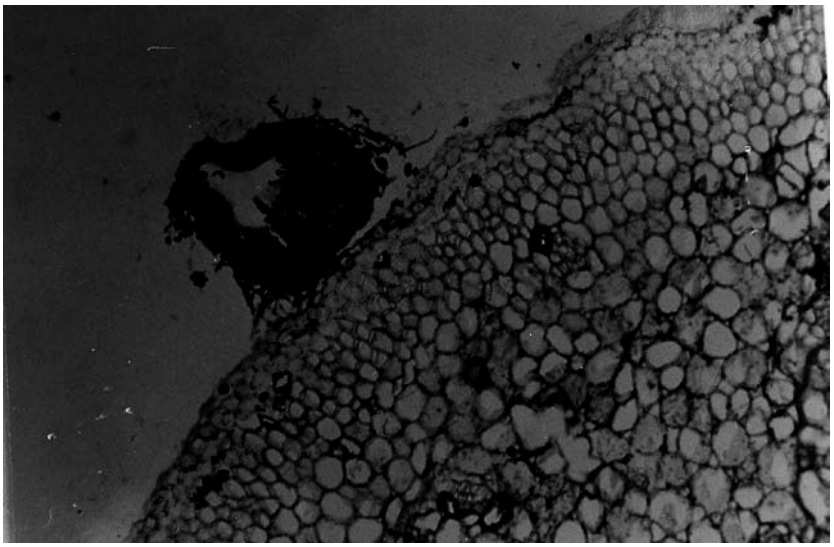

Fig. 2. Transverse section in plumule base of date palm seedling infected with *Coniochaeta velutina* showing the fungus mycelia (stained in blue) in the parenchyma cells of the cortex and the perithecia developed on the surface (x 200).

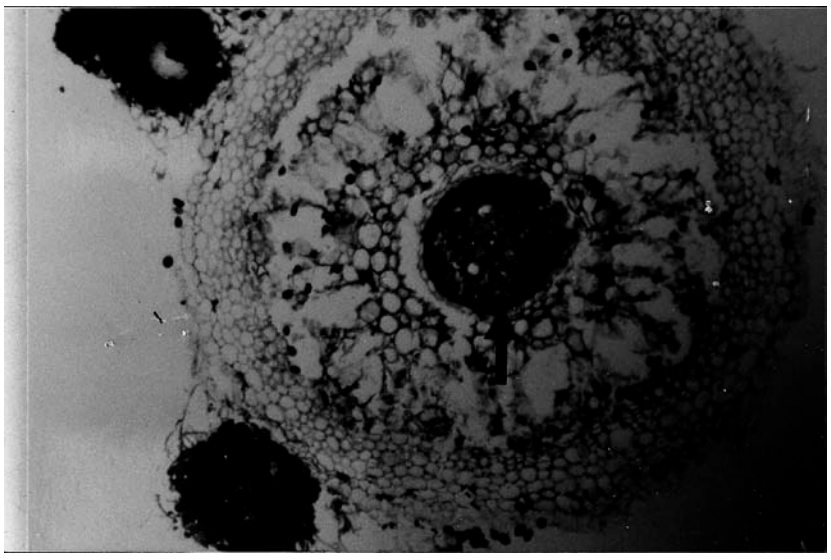

Fig. 3. Transverse section in root of date palm seedling infected with *Coniochaeta velutina* showing the degeneration of the parenchyma cells in the cortex and the presence of the fungal mycelia in the xylem vessels (indicated by the arrow) (x 160).

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تسجيل جديد لوجود الفطر الأسكى *Coniochaeta velutina* كمسبب للفحة أوراق نخيل التمر

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(قدم للنشر في ١٤٢٢/٣/٦هـ؛ وقبل للنشر فى ١٤٢٢/١٠/١٠هـ)

ملخص البحث.تم عزل الفطر *Coniochaeta velutina* (Fuckel) Munk من أوراق نخيل مصابه باللفحه في منطقة القصيم بالمملكة العربية السعودية. ظهرت أعراض اللفحه على الأوراق المصابة في صورة تلون أصفر ذهبي إلى صدأ للوريقات المصابة، يصاحب ذلك وجود الأجسام الثمريه الأسكيه للفطر كنقاط سوداء صغيره بحجم رأس الدبوس على كلا سطحي الوريقات المصابة.

طبقت فروض كوخ للتأكد من القدرة الإمراضيه لهذا الفطر وذلك باستخدام بادرات نخيل صغيره (عمر أربعة أشهر) نامية داخل أنابيب على بيئة صناعية، بالإضافة إلى عدوى أوراق نخيل كبير (عمر خمس سنوات). أظهرت الدراسة التشريحية لقطاعات مختلفة مأخوذة من بادرات النخيل المعداه صناعيا بالفطر *Coniochaeta velutina* أن الفطر ينمو ويتشعب سواء بين الخلايا أو داخل الخلايا البرانشيميه بنسيج القشرة. أمكن كذلك مشاهدة وجود هيفات الفطر داخل الأوعية الخشبية لتلك البادرات المعداه صناعيا.

يعد هذا هو أول تسجيل للفطر *Coniochaeta velutina* كمسبب مرضى على أوراق نخيل التمر.