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Leaf Spot Disease Of Date Palm (Phoenix Dactylfera L.) In Iraq And Its Control.

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ABSTRACT

Date palm (Phoenix dactylifera L.) is one of the most important fruit trees growing in Iraq and represents a good cash crop for many farmers .Leaf spots caused by *Alternaria alternaria* and other fungi have become a threat to date palm s' cultivation in Iraq. *Alternaria* sp was isolated from leaf spots symptoms of date palm leaves when their pathogenicity were tested in laboratory. Polymerase Chain Reaction(PCR) and morphological characters isolates were used to identify leaf spot disease on date palm .Results demonstrated that these isolates belong to *Alternaria* sp based on taxonomic keys. molecular identification of these isolates showed that the isolates belong to *Alternaria* species or species complexes.*Trichoderma harzinum* as a biological control agent against *Alternaria* alternate a causing leaf spot disease on date palm, The findings revealed that the strong reducing effected of *Trichoderma harzinum* towards *Alternaria* alternate can be applied in biological control of this pathogen in vitro and vivio.

Keywords: Leaf spot disease, *Alternaria alternaria*, *Trichoderma harzinum*

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INTRODUCTION

Palm disease are among the major factors the affecting the products. The dominant fungi associated with date palm are: Thielaviopsis paradoxa (Black scorch), Botryodiplodia theobromae (Basal rot), Maugniella scattae (Inflorescence rot), Heminthosporium spp. and Alternaria spp (leaf spots) (Abdalla et al., 2001; Al-sharidy and Molan, 2008). . Leaf spot disease are very common on date palm trees in all date palm growing countries (Carpenter & Elmer,1978; Fayay Mania,2006; El Deeb et al.2007; Livingston et al. 2002) Generally infection is more severe on the lower whorls and old leaves than in upper young leaves, and the infection rate and severity is increased with increasing palm age. Negative correlation between tannin and wax content in the leaves and severity of infection were recorded (Fayad & Mania,2006).

MATERIALS ANDETHODS

Isolation and identification

Samples were collected from various location (BABYLON,DIWANIYAH) provinces were kept in plastic bags and transferred to laboratory for investigation, Individually samples were cuts into small pieces 0.5 cm surface sterilized water and placed potato Dextrose Agar(PDA) medium plates supplemented with 0.5 mg ml-1of streptomycin and incubated for 3-7 days at 28c .Emerge colonies from the tissue pieces were transferred to PDA and incubated at 28c for 10 days. The single spore slants and stored at 4 c .

Pathogenicity test

The inoculum of Alternaria sp isolate was prepared as spore suspension and culture filtrates the method described by Elmeleigi(1986).

The attached and detached date-palm leaflets were divided into three treatments according to the number of the tested sp. isolates and the types of the inoculum used. Each treated detached leaflets were placed in a plastic box padded using wetted and sterilized filter papers. One ml of the spore suspension (10 spores/ml) and/or culture filtrate was placed on the detached leaflets. The boxes were covered with transparent plastic sheets then incubated under laboratory conditions (Hatzipapas., 2002) until symptoms appearance. On the other hand, the attached leaflets were densely sprayed with ten ml of the previously prepared inoculum (spore suspension and/or culture filtrate) using a sterilized atomizer, 100 ml in capacity Alternaria leaf spot symptoms were described and its severity was recorded after three weeks of inoculation according to(Babu etal2004)

DNA extraction

DNA was extracted from pure cultures and from spores obtained directly from infected leaves that had been incubated in a moist chamber for 48 hours to allow abundant spore production The protocols of DNA extraction were done as described (Brillowska-Dąbrowska et al., 2010)

Primers used in this study

The sequences of primers were ITS1: 5': TCCGTAGGTGAACCTGCGG-3' and ITS4:5' TCCTCCGCTTATTGATATGC-3'(White 1990).

PCR amplification was performedusing a Touchgene Thermal Cycler (Barloworld ScientificLtd, United Kingdom). Each 50-µl PCR reaction mixtureconsisted of 36 µl sterile ddH O, 5 µl 10X PCR buffe (Promega), 3 µl MgCl (25 mM), 1.5 µl dNTP (10 mMtotal, 2.5 mM each), 1.5 µl each primer (20 ng/µl), 0.2 µlTaq polymerase (Promega) (5 U/ul), and 1.3 µl templateDNA (20 ng/µl). PCR cycles consisted of an initial denatu-ration step at 94 C for 5 min followed by 42 cycles of 1 minat 93 C (denaturation), 1 min at 30 to 60 C (annealing), and2 min at 72 C (extension). The annealing temperature wasbased on the primer Tm (Table 1), typically five-degree lessthan the lower primer Tm (Tm−5). A final extension cycleat 72 C for 5 min was followed by a 4 C soak. The PCRproducts were visualized with UV light after 1.0−1. 5%agarose-gel electrophoresis in 1X TBE stained with ethidium bromide.

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In Vitro

The antagonistic of Trichorderma harizanium against Aternaria alternaria by Dual culture on PDA media During 5 days the incubation period. Radial growth of pathogen was recorded and percentage inhibition calculated in relation with control by formula .

$$Rr=\frac{R1-R2}{R1}\&100$$

where Rr= Percentage of inhibition,R1=radius of the radial growth of the pathogen towards opposite side in control plat R2=radius of the radial growth of the pathogen towards the opponent antagonist in test plate

***In Vivo* test**

The experiment was conducted in date palm trees(Babylon province. All palm trees used in this study showed high disease severity (as a nature infection) The 3rd whorl leaves from the bottom of tested trees were selected and labeled. Three trees were used as a replicates for each treatment. Tricodermia harizinum were used. 100 ml of the Trichoderma spore suspension was sprayed on each palm once a month starting from march. to July 2018.Control palms were sprayed with water only.The disease severity ranged from 28.2-35.7 at the initiation of spray. disease severity was calculated using the following formula (James, 1971):

$$\text{Disease severity}=\frac{mean\ of\ plant\ tissue\ infected}{mean\ of\ total\ area\ of\ leaflet}$$

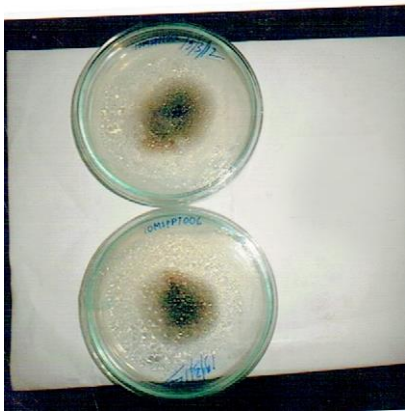
RESULTS

Fungus colonies were dark to gray-black and conidiophores arising singly or in small groups produced spores in chains. Hyphae were brown with light brown conidiophores at the top of each branch. Conidiophores produced conidia.

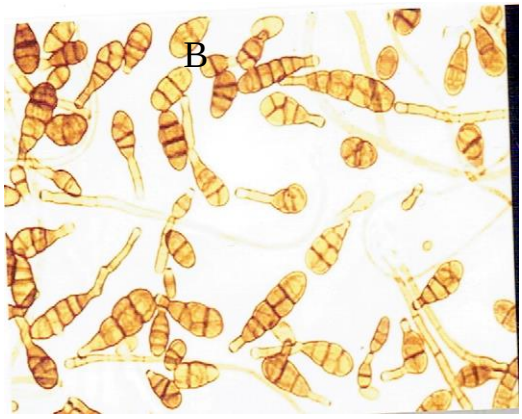
Conidia were oval or ellipsoidal with 3–5 transverse septa and 0–3longitudinal septa, 18.8–29.6 lm in length and8.2–12.1 lm in width (Fig. 1a and b). Based on these morphological characteristics and the description of Alternaria (Woudenberg et al. 2013)

The results of the pathogenicity test showed the ability of Alternaria sp to cause injury and appearance of distinctive symptoms of disease on the leaflet treated.

Figure 1: single spore isolates colony shape B A PORE



A



B

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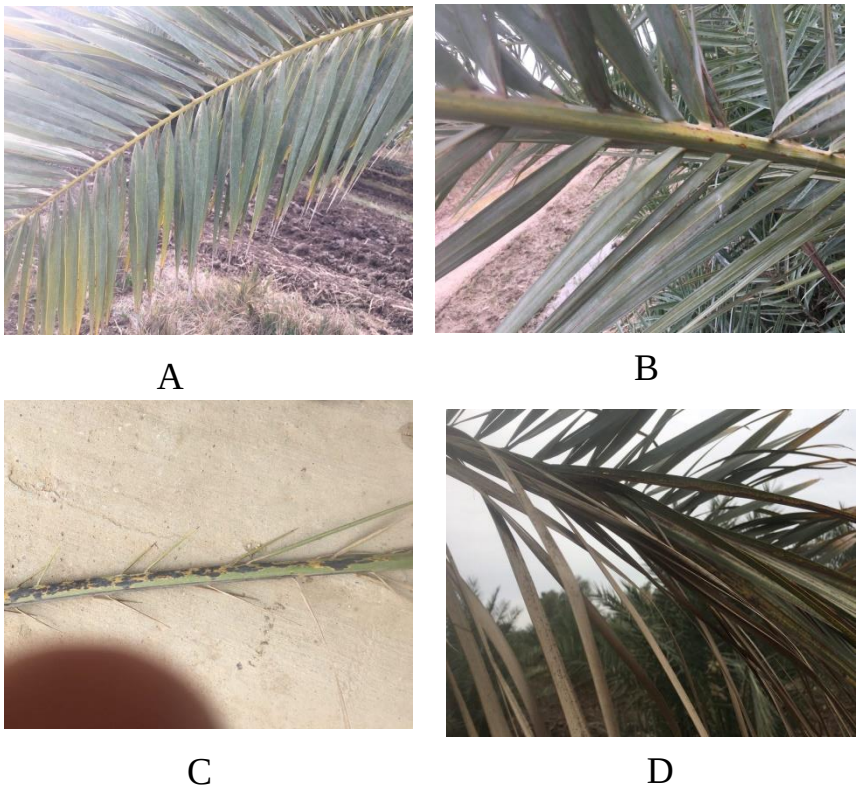
Figure 2: Leaf spot disease symptoms cause by Alternaria alternate



Molecular identified

The results of molecular characterization of Alternaria sp emphasizing on the ITS region of ribosomal DNA(rDNA) with ITS1 and ITS4 primers revealed that the ITS sequence analysis had a 99% of identify with atotal of 305 bp for Alternaria alternaria (Figure3).

Figure 3: PCR amplicons of DNA from leaf spot date palm with ITS primers Aiternaria alternate (305bp)

***In vitro* test**

The results revealed Trichodrium harzianum was shown inhibition of 60.71% over Alternaria alternaria , Where Growth of Trichodrium harzianum and Alternaria alternaria 28(mm),11(mm) respectively. the present study indicated the power for the antagonistic property of T. harzianum against A. alternate

Table 2 Antagonistic activity of Trichoderma harzianum against Alternaria altrnaria

in vitro

Growth of Trichodrium harzianum on PDA	Growth of Alternaria alternaria on PDA	Inhibition %
28 mm	11mm	60.71%

Biological control of leaf spot by Trichodrium harzianum

The results appeared *Trichodrium harzianum* decreased the severity of the leaf spot disease in the field 3-6%. compared to control The effective action trichoderma spp. Against the fungal pathogens thought to be due to the mycoparasitism, antifungal substances and lytic enzymes (Harman,2004) our results concluded that the tested Trichodrium harzianum reduced to growth of pathogen .

DISCUSSION

The present study showed higher spread of fungal diseases on date palm trees in the surveyed area because of the wet conditions and high temperature for most of the year. The results indicated that leaf spots is widespread but high incidence of such diseases is expected especially in the absence of any control measures which may represent a real problem for date palm cultivation in future. The treating time of the tested biological control is important in reducing *Alternaria* leaf spot severity .*Trichodermia harzianum* was highly suppressed and showed over growth on alternaria leaf spot . The disease severity decreased significantly in palms treated with *Trichoderma harzianum Trichoderma harzianum* (Biocont-t) was evaluated against the leaf spot date palm) because of safety in their use of human beings Environmental and bio-biological components . *Trichoderma harizanium* is known as an eco-friendly bio-control agent. It is used effectively to control a wide range of fungal diseases in all types of crops.

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