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Isolation, Identification and Characterization of *Fusarium oxysporum*, the Causal Agent of *Fusarium* Wilt Disease of Date palm *Phoeniex dactylifera* L. in Northern State, Sudan

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A B S T R A C T

Fusarium oxysporum is an important pathogen causing wilt disease in date palm trees. Isolated from infected date palm trees (Rachis, xylem tissues and roots) based on morphological characteristics. Symptoms were reproduced by dipping seedlings roots in pathogen suspension. This study reports for the first time the presence of *Fusarium oxysporum* in Sudan causing the Fusarium wilt disease in date palm trees.

Introduction

Date palm (*Phoenix dactylifera* L.) is one of the oldest fruit trees mentioned in the Holy Quran. Its role as an income generator because of it is high nutritive and social values well recognized in the Arabian and some neighboring countries. It constituted there the basis of survival for many tribes in the desert regions, which still holds to be true today. Date palm production constitutes 70% of exports in the Northern state of Sudan (Dirar, 2003). In Sudan date palm is important fruit tree in the Northern part of the country, where it has been cultivated for more than 3,000 years, with an estimated of about 400 current varieties (Bashab, 2011). Its cultivation is confined to the northern regions, particularly along the Nile banks between latitude 15 : 5 and 22N. Climatically conditions prevailing in north of the country along hot summer cool dry winter scarcity of rains, makes an ideal location for date palm tree cultivation (Osman,1977).

Earlier disease surveillance concerned on disease diagnosis and symptomlogy in infected trees only two identified disease based on isolation, of the causal agent, were black scorch caused by *Thielaviopsis paradoxa* and inflorescences rot caused by *Mauginiella scaettae* (Mohamed, 2003).

The former disease was observed from (Abudoom, Elkuru, samaarat, Elgaba and

Eltolibonab). While the later was reported from Tangasi samaarat.

The objectives of this study were to identify the causal agents of wilt of date palm *Phoenix dactylifera L.* observed in Northern state Merowe locality.

Materials and Methods

Preparation of plant material

Five samples from infected date palm *Phoenix dactylifera* L. trees (roots, rachis, xylem tissues) were collected in paper bags and brought to the laboratory for the isolation and identification of the causal agents.

Isolation of the causal agents

Isolation was done from diseased tissues such as roots, leaves and rachis. Plant materials were washed in tap water to remove adhering soil particles, cut into small pieces of about 0.3cm long, surface disinfected for 2min in for the same period and left to dry on sterilized filter paper and then plated on petri dishes containing water agar (WA) medium. The samples that showed wilt symptoms were plated in peptone penta chloro benzene agar (PPA) medium then sub cultured in carnation leaf agar (CLA) medium in order to obtain uniform size of micro, meso and macroconodia.

The growing fungus was then sub cultured on potato dextrose agar (PDA) medium to obtain high mycelia density and the production of chlamydospores. The cultures were incubated at 25-30^{0}C and the emerging fungi were examined and identified based on their morphological and cultural characteristics (Lesile and summerell, 2006).

Pathogenicity test

The pathogenicity test was conducted under laboratory conditions. Seeds of the two date palm varieties Barakawi and Mishrig Wad Khateeb, were surface sterilized for 2 min with 0.5% sodium hypochlorite soaked in SDW, then washed with SDW before sowing in plastic pots containing sand and clay at 1:2 ratio (five seeds of each cultivar) replicated five times. The pots were kept in the nursery. Conidial suspension of the fungus was prepared by mixing the fungal culture in sterile distilled water in 500 ml flask, shaked well and passed through two layers of filter paper. Two months old plants were inoculated with 2 ml of inoculums suspension, where seedlings roots were separately dipped for one hour in the inoculum suspension at spore conc. 10^6/ ml. The inoculated seedlings were covered with transparent polyethylene sheet for 72 hours.

Results and Discussion

Symptoms of wilt disease

The main visible external symptoms starts with leaf lets whitening below the second row of the middle crown figure (1). The disease progresses from the base of the frond upward to the apex and then proceeds on the dorsal side of the leaf lets from apex to base and ultimately the whole frond is withered figure(2). Such symptoms were found to be similar to those previously reported for Fusarium wilt bayoud disease (Zaid, 2002; Sedra, 1998 and Djerbi, 1983)

Internal disease syndrome observed in a cross and longitudinal sections of rachis and petioles appear as brown pigmentation of xylem tissues figure(3). In acute cases of infection gummy exudates from the destroyed frond occasionally seen in variety Barakawi. The whole date palm tree dies

between 2-36 months figure (4). However, in Morocco this event occurs in 4-24 months (Sedra, 1998).

Fig.1 Whitening of the second row of the heart

Fig.2 whiting of leaf lets in one side

Fig.3 Rachis and petioles longitudinal and horizontal section showing xylem discoloration

Fig.4 *Fusarium oxysporum* microconidia

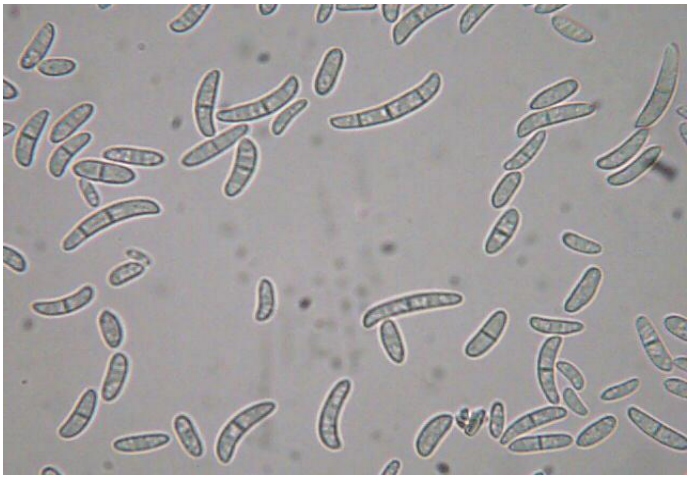

Fig.5 *Fusarium oxysporum* mesoconidia

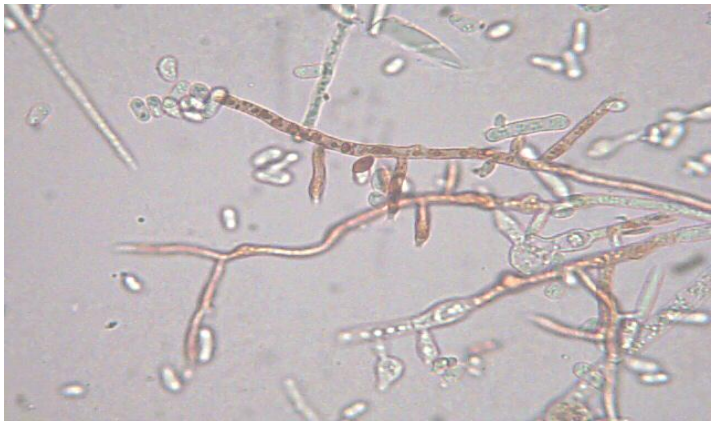

Fig.6 *Fusarium oxysporum* macroconidia

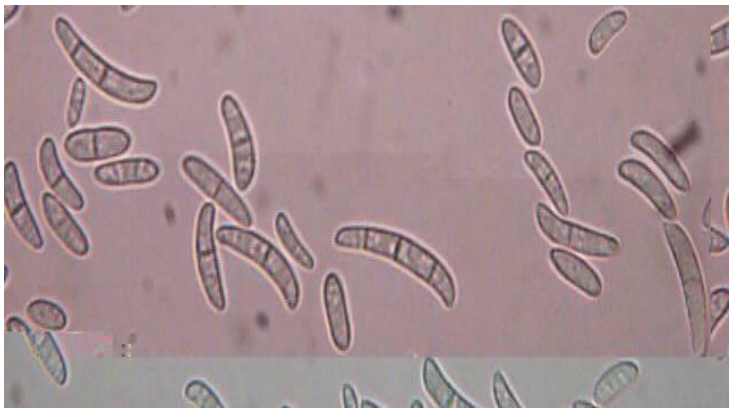

Fig.7 Infected and healthy Wad Khateeb seedlings

Isolation and Identification of the causal agent

The identification of the fungus isolate was based on *in vitro* culture characteristics on Potato Dextrose Agar (PDA) and morphology (micro, meso, macro conidia and Chlamydiospores shape and size) under the microscope (Ellis, 1971).

Morphological studies of the causal agent was iniaitialey carried with peptone Penta chloro nitro benzen Agar or Nash-Snyder medium (PPA) then cultured in Potato Dextrose Agar medium (PDA) showed whitish mycelium grown within four days of incubation at 26-28C°.

The mycelium colony gradually changes to pinkish and later to purple after 15 days. *In vitro* Lab investigation using carnation leaf agar medium (CLA), showed great numbers of hyaline uniformed micro, meso and macro conidia vary in form and dimension on the same culture. Micro conidia are often two celled measuring 5.36-10.9x3.1-4.0µum and occasionally unicellular figure(4) whereas mesoconidies are rarely seen as narrower and longer unicellular attach to the vegetative mycelia with short conidiophores and measure 3.26-8.9x1.3-1.0µm.figure(5). On the other hand the macroconidia are often seen as slightly curved crescent shaped with short almost blunt tip ends, with 3-5 septa and measure 8.26-14.9x5.1-6.27 µm figure (6). The chlamydospores have ring shaped thick walled cells grouped in chain of 2-4 cells.

Pathogenicity test

The early symptoms observed on the leaves of the inoculated seedlings of the date palm varieties Barakawi and Mishrig wad khateeb appeared as brown spots, which later resulted in drying and general death of the seedling figure7. Koch's postulates of disease pathogenicity were satisfied no symptoms were observed on the control plants. The fungus was consistently re-isolated from the inoculated seedlings.

Based on cultural characteristics pathogenicity and biological performance of the isolated fungus, the causal agent was identified as *Fusarium oxysporum* (Lesile and Summerell, 2006) constituting by that the first report of Fusarium wilt disease on date palm in the Sudan.

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