

Isolation and identification of fungal pathogen from *Phoenix dactylifera* (date palm) tissues in Eltaweel oasis in Northern Kordofan, Sudan

Nazik Abdelrhman Mohamed^{1*}; Muna Abelaziz Mohamed²;
Osman Khalil³ and Ismaeel Gibrel³

¹ Faculty of Science, University of Kordofan.

²Institute of Environmental Studies, University of Khartoum.

³ Faculty of Science, International University of Africa.
nazikabdo12@yahoo.com

ABSTRACT

Fusarium oxysporum and Fusarium solani were isolated following standard methods from all infected parts of diseased Date palm (*Phoenix dactylifera*) leaves. The pathogenicity tests on three weeks offshoot proved that *F. oxysporum* and *F. solani* were the most destructive fungus on leaves. Old leaves at lower part susceptible to fusarium wilt. Total discolouration of leaflet partial or on one side of the rachis was noticed. Desiccation or death was found to be associated with a shade of brown in one site leaflets whereas the opposite sites of the rachis were healthy.

Keywords: *Fusarium*, micronidia, macronidia, chlamydo spores, leaves.

INTRODUCTION

Fusarium spp. are Hypocreales Ascomycota (Moretti, 2009; Davis *et al.*, 2010). The genus *Fusarium* comprises a high number of fungal species that causes diseases in several agriculturally important crops (Garofalo *et al.*, 2003). Many of *Fusarium* species producing a wide range of biologically active secondary metabolites (e.g. mycotoxins) with extraordinary chemical diversity (Moretti, 2009; Davis *et al.*, 2010; Garofalo *et al.*, 2003).

Infections of date palm by *Fusarium* can occur from germinating seeds up to mature vegetative tissues, depending on the host plant and *Fusarium* species involved (Elliott, 2006; El-Deeb *et al.*, 2006; Armengol *et al.*, 2005). Therefore, it has to be identified accurately and as early as possible to predict the potential toxicological risk to date palm trees, and to prevent toxins entering the food chain (Elliott, 2006; El-Deeb *et al.*, 2006; Armengol *et al.*, 2005).

MATERIAL AND METHODS

The infected wooden parts were collected for identification of the fungus damage. The standard techniques set by Armengol *et al.*, (2005) were followed for isolation and identification of fungal pathogen from date palm.

1. Sterilization of glass ware and steel tools

Petri dishes, forceps, scalpels, scissors were sterilized by using hot air oven at 180 °C for one hour. These tools were kept sterilized until used.

2. Preparation of culture media for growing of fungi

Potato dextrose agar (PDA) and Sabouraud dextrose agar (SDA), HiMedia laboratories Pvt. Ltd India, were used for cultivation and isolation of pathogenic fungi from the date palm leaves with black spots due to fungal infection. The first culture medium (PDA), (HiMedia laboratories Pvt. Ltd, India) was prepared by dissolving 39 g of medium powder in 1000 ml distilled water. The mixture was boiled in a

water bath to melt the agar at 100 °C for 15 minutes. Then the flask containing the rehydrated medium was plugged properly with cotton wool and sterilized by autoclaving at 15 pound pressure for 20 min. After autoclaving the sterilized medium was cooled and kept at 45 °C in a water bath, and then it was poured into sterilized petri plates under aseptic conditions in a laminar flow beside Bunsen burner flame. Each plate contained about 15-20 ml of liquid agar; plates were allowed to solidify at room temperature and kept sterilized at 4 °C until used. The second culture medium was (SDA), (HiMedia laboratories Pvt. Ltd, India). It was prepared by the same procedure described above and medium plates were kept sterilized at 4 °C until used.

3. Collection of samples

Discoloured dead leaves of date palm with black spots on surface (Fig. 1) suspected to be infected with fungal pathogen were collected randomly from different date palm trees in Northern Kordofan state, Bara Province. Collected samples were brought to the laboratory in sterilized box and kept dry at room temperature until used.

4. Isolation of fungi from Date Palm leaves tissues

Collected leaves samples were cut into small pieces with sterile scissor. Surface sterilization was done to remove surface contaminants spores by washing in 95 % ethanol for one minute. After alcohol washing the pieces of leaves were transferred by sterile forceps into petri dishes contained sterile deionized water and washed gently three times after that the pieces were placed on PDA and others on SDA. The cultured plates were incubated at 25°C for 4 days after the end of which the fungus growth was noticed Figs. 2.

5. Purification of isolated fungal colonies

Pure cultures were obtained from the mixed isolated colonies by picking up a part from each colony using sterile forceps and transferred into new petri plates containing PDA medium. Nine colonies have been sub cultured and the plates were incubated at 25 °C for 4 days after which isolated fungal colonies were ready for identification studies. The identification was done on the basis of morphological and cultural features following Ellis *et al.*, (2007).

6. Microscopic examinations for isolated fungi

Wet mounting preparations from each colony were made by removing a small piece of hyphae using sterile needle and mixed with a drop of lacto phenol cotton blue dye on glass slide. A cover slip was applied over each slide before examined under compound microscope. Another wet mounting technique was used. In this technique adhesive plaster method, a piece of adhesive plaster was attached above the mycelia colonies removing some fungal

structure, then applied to slide on which lacto phenol cotton blue was dropped. Slides were examined under microscope. Macro and micro conidia characteristics of the hyphae, presence or absence of septation and the shapes of spores were recorded for identification of the isolates.

7. Re-infecting the date palm tissues with isolated fungi

An experiment was applied in order to confirm the pathogenicity of the isolated fungi. Selected date palm trees in the yard of Faculty of Science, University of Khartoum were inoculated with isolated fungi. Inoculation was done by cleaning and disinfecting surface of young green leaves with 70 % ethanol. Small area in the middle of the leaf was scratched by sterile scalpel. A small part from each colony was removed by sterile forceps and inoculated onto scratched areas and covered with sterile foil. The whole inoculated leaves were covered with wet plastic sack sealed to leaflets with adhesive plaster to maintain moisture inside the inoculums. The infected leaves were left for three weeks for developing fungal pathogen as recommended by Koch's postulates.

RESULTS

In this study several date palm trees in the oases showed symptoms of a fungal disease caused by *Fusarium* wilt or vascular wilt and there are two species of *Fusarium* responsible for disease such as: *Fusarium oxysporum* and *Fusarium solani*.

Old leaves at lower part are susceptible to *Fusarium* wilt. Total discoloration of Leaflets partial or on one side of the rachis was noticed.

Desiccation or death was found to be associated with a shade of brown in one site leaflets whereas the opposite sites of the rachis were healthy.

1. Macroscopic characters

The major macroscopic morphological characteristics including growth time, colonies colours and sporulation conditions were recorded and used in identification of the isolates in the present study. Colonies growth on (Potato dextrose agar) PDA medium was obtained after 4 days incubation at 25 °C. Nine colonies were grown; they showed different colours that vary from white, pink, grey, green-purple and beige on obverse side. Colours were most orange and dark grey on the reverse side. The morphological characteristics of the isolates are presented in Table (1) and Fig. 3.

2. Microscopic characters:

Microscopic examination was applied to isolate by mounting a piece of hyphae with a drop of lactophenol cotton blue on slide and covered with slips and examined under microscope. Results of examination revealed that most observed features were septation of the hyphae in all isolated colonies and formation of macro and micro conidia spores as shown in Fig. 4.

In some isolates chlamydospores with curved spindle shape were found in Fig .5. This is one of unique characteristics of *Fusarium* spp. identification. The isolates features in the present study matched features of two *Fusarium* species previously described and displayed by (Ellis *et al.*, 2007). Also similar evidences are offered by the web site [www.mycologyonline](http://www.mycologyonline.com), where the fungus species is supported with photos of the septated hyphae, colours of colonies, shape of macro and micronidia and chlamydospores.

The morphological features of *Fusarium oxysporum* indicated while coloured, macroconidia is fusiform slightly curved and pointed at the tip mostly three septate. Microconidia are abundant, not in chains, ellipsoidal to cylindrical, straight or often curved. The morphological features of *Fusarium solani* showed rapid growth of the colonies in less than 4 days. Macroconidia are formed from multi-branched conidiophores. With 3- to 5 septate, fusiform often moderately curved. Micro conidia are usually abundant, cylindrical to oval. Chlamydospores are hyaline globes, borne in single or in pairs on short lateral hyphen branches.

DISCUSSION

The present study, found that *Fusarium oxysporum* and *Fusarium solani* are the causative agents affecting date palm trees in oases of Northern Kordofan. These results agree with Logan and El Bakri (1990) who isolated the same genus *Fusarium moniliforme* from Dongola State. The observed symptoms on the surveyed date palm trees in the study area included the appearance of black spots and scales on the leaves surfaces which indicated that fungal pathogen was the cause agent of disease case. The isolated pathogenic features in the present study, matched the features of two *Fusarium* species previously described by (Ellis *et al.*, 2007). Confirmatory evidences were the similarity of the septated hyphae, colours of colonies, shape of micronidia and macroconidia and the shapes of chlamydospores, with those published by the web site [www.mycologyonline](http://www.mycologyonline.com).

Morphological features of *F. oxysporum* isolates indicated that they grow rapidly in 4 days, its colonies are white sized about 4.5 cm in diameter, macroconidia is fusiform slightly curved and pointed at the tip mostly three septate, microconidia are abundant, not in chains, ellipsoidal to cylindrical, straight or often curved. This is in accord with

Garofalo and McMillan, 2003; Armengol *et al.*, 2005; El Deeb *et al.*, 2006; Moretti, 2009; and Ammar and El-Naggar, 2011. Colonies of *F. solani* grow rapidly in 4 days, its macroconidia are formed from multi-branched conidiophores with 3 to 5 septate, fusiform and often moderately curved. The Microconidia are usually abundant, cylindrical to oval. Chlamydospores are hyaline globes, borne in single or in pairs on short lateral hyphen branches. The morphological features recorded during this study are in accord with (Armengol *et al.*, 2005; El Deeb *et al.*, 2006 and Moretti, 2009). Several fungal diseases of date palm trees have been reported from many date producing countries but *F. oxysporum* and *F. solani* are the most serious fungi causing diseases to the trees (El Deeb *et al.*, 2006).

Acknowledgements

We are grateful to Prof. Zuheir N. Mahmoud for critically reading the manuscript.

References

- Ammar, I.D. and El-Naggar, A.M. 2011. Date Palm (*Phoenix dactylifera* L.) Fungal diseases in Najran, Saudi Arabia. *Int. J. Plant Path.* 2:126-135.
- Armengol, A., Moretti, A., Perrone, G., Vicent, A., Bengoechea, J. and Jimenez, J. 2005. Identification, incidence and characterization of *Fusarium proliferatum* on ornamental palms in Spain. *European J. Plant Path.* 112:123-131.
- Davis, R.M., Holesmes, E.A., Colyer, P.D. and Bennett, R.S. 2010. Molecular genetics classification of *Fusarium oxysporum* F. sp. *Vasinfeculum*. Beltwide Cotton Conferences, New Orleans, Louisiana, United States of America January 4-7.
- El-Deeb, H.M., Arab, Y.A. and Lashin, S.M. 2006. Fungal disease of date palm off-shoots in Egypt. *Pak. J. Agri., Agril. Eng, Vet. Sc.* 22: (2).
- Elliott, M.L. 2006. Leaf Spots and Leaf Blights of Palm. Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida, Gainesville, FL. pp-218.
- Ellis, D., Davis, S., Alexiou, H., Handke, R. and Bartley, R. 2007. Descriptions of Medical Fungi. Mycology Unit, Women's and Children's Hospital North Adelaide, Australia. pp.68-70.
- Garofalo, J. and McMillan, R.T.Jr. 2003. *Fusarium* wilts of date palms in south Florida. Miami-Dade County/ University of Florida Cooperative Extension Service.
- Logan, J.W.M. and El Bakri, A. 1990. Termites damage to date palms (*Phoenix dactylifera* L.)

in Northern Sudan with particular reference to the Dongola District. Trop. Sci.30:95-108.

Moretti, A. N. 2009. Taxonomy of *Fusarium* genus, a continuous fight between lumpers and splitters. Proc. Nat. Sci., Manteca Ruska Novi Sad, No-117:7-13.

www.mycologyonline

Table. 1. Morphological features of the isolated fungi

Colour on		Size of colony (cm)	Shape of spores	No. of septation Spores
Obverse	Reverse			
Pink	Orange	4	Ovid	0
Pink with green ring	Orange	4	Ovid	0
Grey	Dark purple	6.5	Ovid	0
White	Bright brown	6	Ovid + fusiform	0
Dark green	Black	5.5	Ovid	0
Pale pink	Dark pink	3.5	Ovid	0
White pinkish	Orange	4.2	Fusiform	3
Creamy-pink	Orange	2.8	Ovid	0
Light brown	Dark orange	5.4	Ovid	0

Figures



Fig.1. Discoloured dead leaves of a date palm with black spots of *Fusarium* sp.

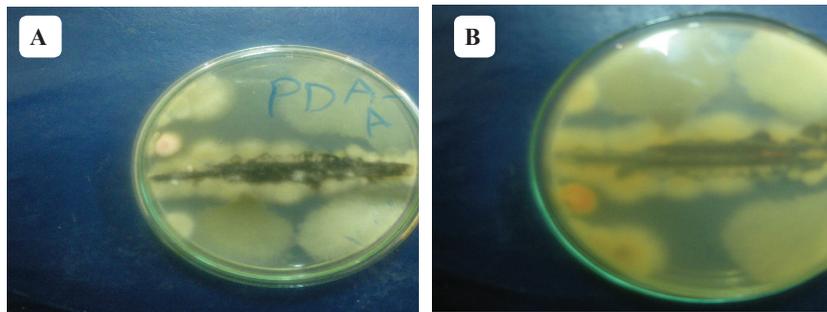


Fig.2. Fungus growth after 4 days incubation at 25°C. Obverse side (A), reverse side (B).

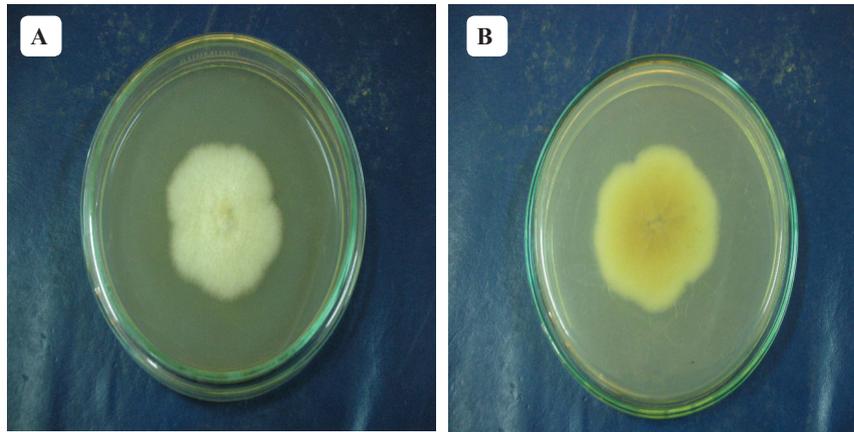


Fig. 3. Morphological characters of isolated fungi obverse side (A) white colour and Reverse side with yellowish colour (B).

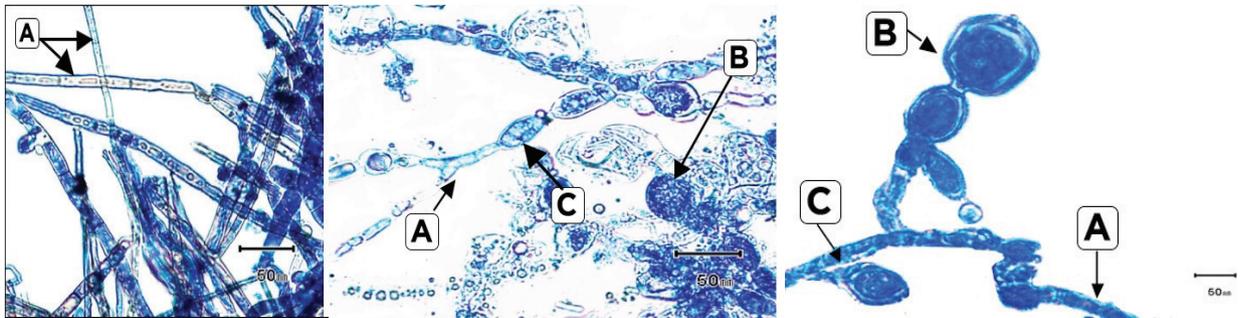


Fig . 4. Formation of mycila (A), macro-conidia (B) and micro-conidia (C) form of *Fusarium* sp.

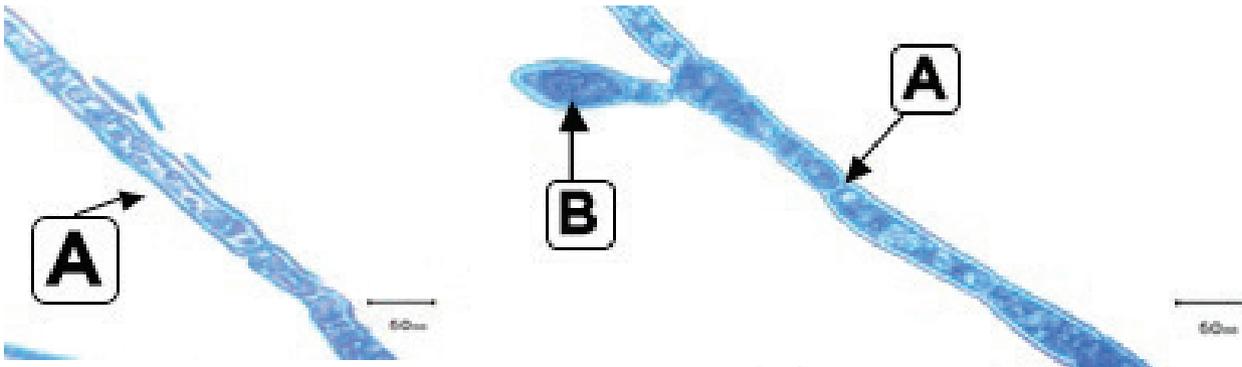


Fig . 5. Formation of mycila (A) and chlamydo spores (B) with curved spindle shape.

