JOURNAL OF PURE AND APPLIED MICROBIOLOGY, Sept. 2016. Vol. **10**(3), p. 2467-2470

First Report of Wilt Disease Caused by *Fusarium oxysporum* spp on Fishtail Palm in India

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(Received: 07 February 2016; accepted: 11 March 2016)

The identification and characterization of *Fusarium oxysporum* sp isolated from vascular tissues of root of Fishtail (Caryota urense) palm from Bastar district of Chhattisgarh state. The fungal species produced hyaline mycelium and produced spores within the host tissues as well as in the culture. Micro conidia were most abundantly produced and observed as small, elliptical or curved unicellular or with 1-2 septa and measured 5-15 x 2-4 um. Macro conidia were produced in small cushion of somatic mycelium measured long, curved septa 3-4 and size 15-25 x 3-5 um. Chlymydospores was also formed in the host as well as in old culture. They developed from any cell of the fungus. The fungal growth on plate was filamentous and whitish in color. The results of molecular analysis revealed that the ~500 bp rDNA of *Fusarium oxysporum* sp from fishtail shares 100% sequence identity with that of known Fusarium oxysporum sp isolates. The identified species proved its pathogenicity characterized on aerial part of the plants as symptoms were observed in any one leaf blade of plant dried initially characteristically as yellow to brown colored, drying of leaves started from margin. Infection was also observed below the ground level as brown to dark black colored vessels.

Keywords: fishtail palm, *Caryota urens* L., wilt, *Fusarium*, sulphi.

Fishtail palm (*Caryota urens* L.) belongs to the family Palmaceae. It is one of the most popular ornamental palms grown in home stead garden and road side. However in Chhattisgarh state of India, it is grown for economical and social purpose and is locally called "*Sulphi*". The sap extracted from spath (inflorescence) and it is one of the most important sources of income earning (Figure 2 a). In view of its socio-economic importance, Fishtail palm is blended with culture of tribal society as its nutritious sap is offered in all social meetings and rituals. The fresh sap taste like juice and fermentation starts quickly and in a few hours it becomes alcoholic. The juice is tapped from the spadix before they are completely developed. The flowers emerged after 8-14 years in dwarf genotype while 16-20 year in tall genotype of sulphi. The healthy palm produces 2500-3500 liters of sap per spathe (inflorescence) from the time of its flowers production till its death (tapping period 4-7 years). It is estimated that tribal farmer earns approximately rupees 25-30 thousand per year from single plant. Due to its economic importance it is an important palm for the farmers of Bastar plateau of Chhattisgarh state in India. Recently, these palms are facing wilting problem in large area of chhattishgarh state especially in Bastar region. Sulphi palms are dying in very large number at all stages of its life (Figure 2 b).

All experiments were conducted at Saheed Gundadhur College of Agriculture and Research Station (IGKV), Kumhrawand, Jagdalpur (Chhattisgarh) under the Adhoc project on "Identification and management of factors responsible for wilt of sulphi palm (*Caryota urens*,

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L.) in Bastar plateau" opted by AICRP on Palms, CPCRI, Kasaragod, Kerala, India

Extensive surveys were conducted for monitoring of wilt incidence in different location of Bastar plateau during the year of 2010, 2011, 2012 and 2013. During the survey period, the average incidence of three year was observed 13.80%. The maximum occurrence of wilt disease was observed just after rainy season (November to February month), while moderate occurred in the rainy and summer season. In all infected palms common noticed symptoms was wilting and finally death of the plant (Bhanwar *et al.,* 2013). The present attempts have been made to identify the pathogen through morphological study, pathogenicity test and gene sequence analysis.

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Query  1    ATCATTACCGAGTTTACAACCTCCCAAACCCCTGTGAACATACCACTTGTTGCCTCGGCG  60
            |||||||||||||||||| ||||||||||||||||||||||||||||||||||||||||
Sbjct  26   ATCATTACCGAGTTTACAA-CTCCCAAACCCCTGTGAACATACCACTTGTTGCCTCGGCG  84

Query  61   GATCAGCCCGCTCCCGGTAAAACGGGACGGCCCGCCAGAGGACCCCTAAACTCTGTTTCT  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  85   GATCAGCCCGCTCCCGGTAAAACGGGACGGCCCGCCAGAGGACCCCTAAACTCTGTTTCT  144

Query  121  ATATGTAACTTCTGAGTAAAACCATAAATAAATCAAAACTTTCAACAACGGATCTCTTGG  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  145  ATATGTAACTTCTGAGTAAAACCATAAATAAATCAAAACTTTCAACAACGGATCTCTTGG  204

Query  181  TTCTGGCATCGATGAAGAACGCAGCAAAATGCGATAAGTAATGTGAATTGCAGAATTCAG  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  205  TTCTGGCATCGATGAAGAACGCAGCAAAATGCGATAAGTAATGTGAATTGCAGAATTCAG  264

Query  241  TGAATCATCGAATCTTTGAACGCACATTGCGCCCGCCAGTATTCTGGCGGGCATGCCTGT  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  265  TGAATCATCGAATCTTTGAACGCACATTGCGCCCGCCAGTATTCTGGCGGGCATGCCTGT  324

Query  301  TCGAGCGTCATTTCAACCCTCAAGCACAGCTTGGTGTTGGGACTCGCGTTAATTCGCGTT  360
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Sbjct  325  TCGAGCGTCATTTCAACCCTCAAGCACAGCTTGGTGTTGGGACTCGCGTTAATTCGCGTT  384

Query  361  CCTCAAATTGATTGGCGGTCACGTCGAGCTTCCATAGCGTAGTAGTAAAACCCTCGTTAC  420
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Sbjct  385  CCTCAAATTGATTGGCGGTCACGTCGAGCTTCCATAGCGTAGTAGTAAAACCCTCGTTAC  444

Query  421  TGGTAATCGTCGCGGCCACGCCGTTAAACCCCAACTTCTGAATGTTGACCTCGGATCAGG  480
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Sbjct  445  TGGTAATCGTCGCGGCCACGCCGTTAAACCCCAACTTCTGAATGTTGACCTCGGATCAGG  504

Query  481  TAGGAATACCCGCTGAACTTAAGCATATCATTAAAGCGGAGGAA  524
            |||||||||||||||||||||||||||||||| ||||||||
Sbjct  505  TAGGAATACCCGCTGAACTTAAGCATATCATTAA-GCGGAGGAA  54
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Fig. 1. Nucleotide sequence analysis of isolated *Fusarium* with known *Fusarium* species

The symptomatology of wilt disease on naturally infected sulphi palms was studied at farmer's field by visual observation. Observations were recorded on roots, leaves and stem. Different types of wilt symptoms were also observed in seedlings of fishtail palms during pathogenicity study. On aerial part of the plant, the typical wilt symptoms was observed as initial discoloration of leaves started from either apical leaves or middle leaves or older leaves. Initial symptom was observed on any one leaf blade of plant characteristically as yellow to brown colored, drying of leaves started from margin (Figure 2 c). In case of developing of disease, symptoms were observed as spread of blight symptoms to another leaves later on severe infection whole plant was dried. Infection was also observed below the ground level as brown to dark black colored vessels (Figure 2 d). The hyphae enter into the xylem vessels and plugged with hyphae. In severe infections roots of wilted palm become water soaked and soften. In advance of disease roots become rotted and contaminated by some secondary fungus (e.g.; *Aspergillus, Bionacteria, Rhizoctonia* etc.), bacteria and nematode (Figure 2 e). Such type of symptoms observed in all partial and complete wilted palms.

Morphological study was carried out with the help of phase contrast microscope. Microscopic observations were taken on different structure of fungi of most responsible fungus and also catch the image of fungi which was isolated and purified. The fungus in the host was confined to vascular tissues and was both inter and intra cellular. The mycelium was hyaline and produced spores within the host tissues as well as in the culture. Micro conidia were most abundantly produced and observed as small, elliptical or curved unicellular or with 1-2 septa and measured 5-15 x 2-4 um. Macro conidia were produced in small cushion of somatic mycelium measured long, curved septa 3-4 and size 15-25 x 3-5 um (Figure 2 f-h). Chlamydospores were also formed in the host tissues as well as in old culture. They developed from any cell of the fungus. The fungal growth on plate was filamentous and whitish in color. Based on the morphological character of the fungus, the causal organism identified as *Fusarium oxysporum*.

Table 1. Nucleotide sequence identity of the DNA among Fusarium spp

Accession	Sequences producing significant alignments: Description	Max score	Query coverage	Max ident
HQ658968.1	Fusarium oxysporum f. sp. Cepae CSC6057	955	100%	99%
HQ658965.1	Fusarium oxysporum f. sp. Cepae CSC6053	955	100%	99%
HQ658958.1	Fusarium oxysporum f. sp. Cepae CSC6007	955	100%	99%
HQ658961.1	Fusarium oxysporum f. sp. Cepae CSC6035	953	99%	99%
HQ658962.1	Fusarium oxysporum f. sp. Cepae CSC6036	950	100%	99%
HM756257.1	Fusarium oxysporum f. sp. phaseoli DM0910191	950	100%	99%
EU750682.1	Fusarium sp. 14018	950	100%	99%
EU073196.1	Fusarium oxysporum	950	100%	99%
EU002984.1	Uncultured Fusarium clone 7g	950	100%	99%
EU002963.1	Uncultured Fusarium clone 4d	950	100%	99%
EF495235.1	Fusarium oxysporum strain Ppf12	950	100%	99%
DQ780422.1	Fusarium oxysporum isolate Endophyte 60	950	100%	99%
AY188919.1	Fusarium oxysporum f. sp. melonis	950	100%	99%

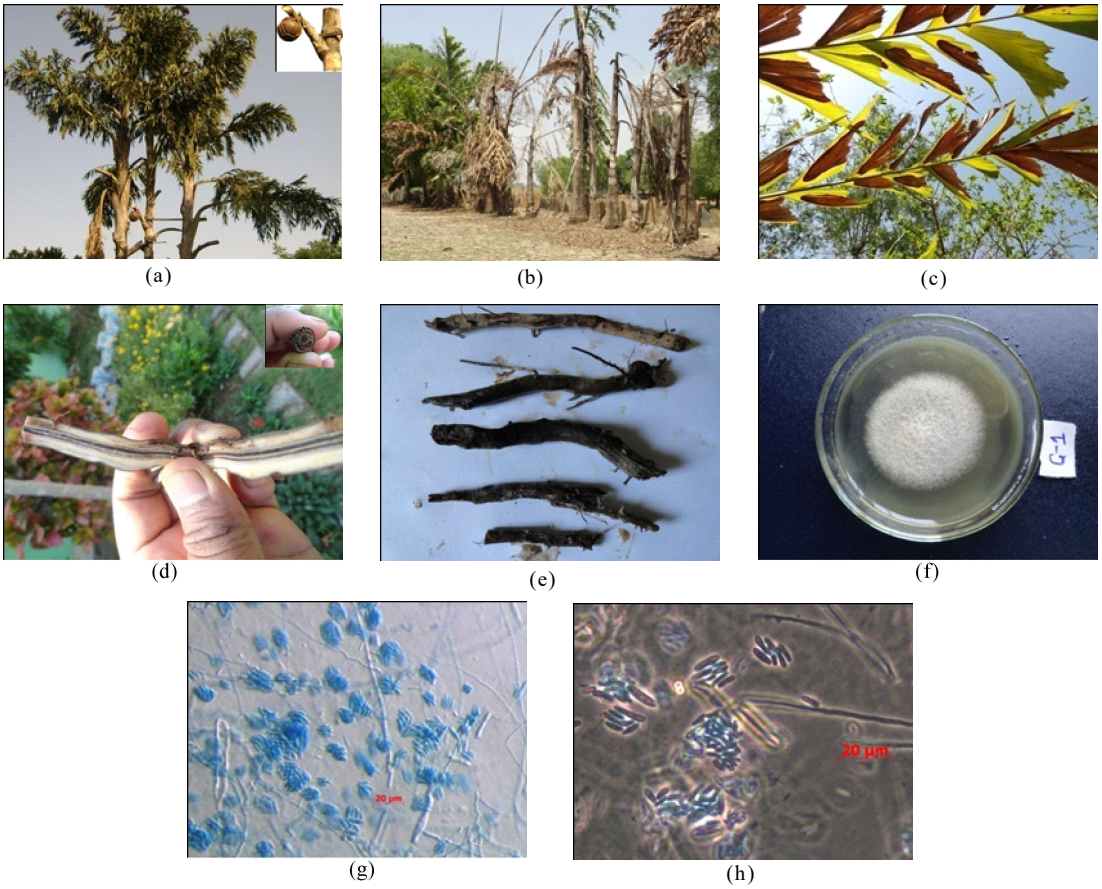

Fig. 2. a= Healthy *Caryota* palm; b=*Fusarium oxysporum* infected *Caryota* palm; c=blight symptom on leaf blade; d=Rotted roots; e=Infected xylem vessels; f= Mycelium of *F. oxysporum* in Petri plate; g-h=Macro and micro conidia

To further support the identified result was amplified by PCR. The ~500 bp rDNA of the most probable isolates was sequenced for the sequence analysis (Figure 1). The genomic DNA was isolated in pure form from the purified fungal culture isolated from roots of infected plant. Nearly ~500 bp rDNA fragments were successfully amplified using universal primers. The sequencing PCR was set up with ABI-BigDye® terminatorv3.1 cycle sequencing kit. The raw sequence obtained from ABI 3100 automated DNA sequencer was manually edited for inconsistency. The sequence data was aligned with publically available sequences & analyzed to reach identity. The entire molecular test was carried out in Agarkar Research Center, Pune, Maharastra. Identification of *F. oxysporum* sp was confirmed by PCR with the universal primers (Table 1), which amplified a fragment of 500 bp rDNA. Similar work done by Plyler *et al*. in 1999. Fungal strain was sequenced and showed 99% sequence similarity with genus *Fusarium* L. The genus currently has 111 species as mentioned in the *Dictionary of Fungi*, 10th edition edited by Paul Kirk *et al*., in 2008.

This work is first time attempted in India and reports *Fusarium oxysporum* spp on *Caryota urens* L. palm. Therefore, it constitutes a new record from India. Similarly, *F. proliferatum* was described as the causal agent of wilt and dieback of date palms in Saudi Arabia (Abdalla *et al*., 2000). More recently, Armengol *et al*. (2005) reported that *F. proliferatum* caused wilt and dieback on *P. canariensis*, *P. dactylifera*, *P. reclinata*, *hamaerops humilis*, *Trachicarpus fortunei*, *Washingtonia filifera* and *W. robusta* in Spain.

ACKNOWLEDGEMENT

This work was supported by special grant funded by Project coordinator, AICRIP, Palms, CPCRI, Kesargod, Kerala. We are also thankful to Dr. S S Rao, Dean, S.G. Colleage of Agriculture and Research Station, Jagdalpur for providing facilities. Thanks also to Director, Agarkar Research Institute, Pune for confirming the identity of fungus.

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