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First Report of Fusarium falciforme inciting Wilt and Root Rot in Malabar neem (Melia dubia) by Polyphasic Characterization from the Bundelkhand Region of Central India

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Abstract

Malabar neem (*Melia dubia* L.) is a fast-growing tree species valued for its pulp wood and timber production in agroforestry systems of India. During 2021 and 2022, we observed a severe wilt and root rot disease that caused 90% mortality of one-year-old *M. dubia* plantations in Jhansi, Uttar Pradesh. A polyphasic approach of etiological investigation revealed progressive chlorosis, defoliation, branch desiccation, root decay and death infected trees. A *Fusarium* isolate MFs1 was obtained from infected roots that produced cottony aerial mycelium, dark purple pigmentation on culture media along with falcate macroconidia, and abundant chlamydospores. Pathogenicity assay by artificial inoculation on healthy seedlings reproduced typical wilt symptoms, fulfilling Koch's postulates. Molecular identification using four genetic markers *ITS*, *TEF1α*, *RPB1*, and *RPB2* identified MFs1 as *Fusarium falciforme* (a member of the *Fusarium solani* species complex). The concatenated sequences showed 100% similarity with reference strains of *F. falciforme* NRRL 32313 and 99.92% with NRRL 28562/28563. Phylogenetic analysis based on the Maximum-likelihood method placed MFs1 distinctly within the *Fusarium solani* species complex in close clustering with members of *Fusarium falciforme*. This is the first report of *Fusarium falciforme* causing wilt and root rot disease in *M. dubia* expanding the known host range of this pathogen. Our findings point to an emerging threat to *M. dubia*-based agroforestry systems and highlight the urgent need for integrated disease management approaches, including resistant planting material and biological control options, to sustain cultivation in semi-arid regions.

Introduction

India is facing a growing gap between industrial timber demand and forest productivity. According to the latest estimates, the total area of forests and trees in the country stands at 82.73 million hectares, covering 25.17 percent of the total geographical area. While there is an increase in 1445.81 km² area of forests and trees from 2021 (India state of Forest Report (ISFR) 2023), the actual productivity in terms of average annual growth is only between 0.5 to 0.7 m^3 ha^{-1} far below the global average 2.1 m^3 ha^{-1}. This suggests that the industries rely on biomass energy, plywood, furniture and pulpwood struggle to secure enough raw material (Srivastava 2005; Parthiban et al. 2011). Furthermore, global timber consumption is growing by 1.7% annually (South 1999), and it is estimated that timber demand increased from 58 million cubic meters to 158 million cubic meters over the 15-year period from 2005 to 2020 (Saravanan et al. 2014). The recent projections estimate that by 2030 we will need between 153 and 183 million m³ of timber, but domestic supply is expected to meet only about 60 million m³ (Parthiban et al. 2011, 2021; Rajkumar 2025). Due to the low natural forest productivity and legal restrictions wood-based industries are heavily depended on agroforestry, farm forestry and other non-forest resources with major leap of 22.47 million m3 in the potential production of industrial wood as compared to the estimates reported in ISFR 2017 (Parthiban et al. 2021; ISFR 2023; Jose et al. 2024; Mutune et al. 2024; Prateek 2025).

Malabar neem (*Melia dubia* Cav.) is one of the alternative species that potentially contribute to demand for timber, plywood, pulp, and fuel (Parthiban et al. 2009). It is a native species of Southeast Asia and

Australia and in India, it occurs naturally between 600 m and 1,800 m above sea level, especially in the foothills of Sikkim Himalayas, North Bengal, Assam, Western Ghats, Deccan Plateau, Khasi Hills and Orissa (Gamble, 1992). Due to its high adaptability, it can be successfully cultivated across diverse agro-climatic zones of the country (Shah et al. 2016). With a rotation period of 6–12 years, *M. dubia* is ideal for agroforestry and farm forestry, and is gaining increasing economic importance in both domestic and international markets. Productivity estimates range from 80 to 150 t ha^{-1} yr^{-1} of biomass over 4–8 years, and saplings may reach 12 m in height within two years (Chavan et al. 2022). The escalating commercial demand for *M. dubia* has intensified the need for robust varietal improvement, Quality Planting Material production and disease management.

Despite its vigour, *M. dubia* is vulnerable to various infections throughout its life cycle. A diverse spectrum of pathogens that affect all growth stages of *M. dubia*, have been recorded from nursery to harvest (Mohanan 2001; Muthulakshmi et al. 2016; Ashajyothi et al. 2021a; Muttappagol et al. 2025) by various fungal pathogens such as *Fusarium solani*, *Colletotrichum dematium*, *Cylindrocladium ilicicola*, *Diaporthe phaseolorum, Botryodiplodia theobromae*, and *Lasiodiplodia theobromae*, *Fusarium moniliformis*, *Phoma* sp., and *Pythium* sp. (Muthulakshmi et al. 2016; Ashajyothi et al. 2021; Krupalini and Janardhana 2024; Muthulakshmi and Pradipa 2025; Muttappagol et al. 2025). Recent molecular diagnostics have further uncovered the complexity of *M. dubia* pathosystems, exemplified by the discovery of mixed infections involving *'Candidatus* Phytoplasma asteris' (16SrI-B and 16SrI-X subgroups), chilli leaf curl virus, and tomato leaf curl Bangladesh beta satellite, highlighted the role of emerging and synergistic biotic agents in *M. dubia* disease etiology (Muttappagol et al. 2025).

However, *Fusarium* species have emerged as a significant constraint in *M. dubia* cultivation whose severity was demonstrated during a 2016 outbreak at the Forest Research Institute (FRI), Dehradun, India where approximately 100,000 seedlings were lost to severe wilt and root rot, incited by *Fusarium solani*. Similar wilting was reported from Haryana and Uttar Pradesh, India nurseries during 2012 and 2021 (Pandey et al. 2018; Ashajyothi et al. 2021b) which confirmed widespread occurrence of the disease being a persistent threat across northern Indian nursery systems (Pandey et al. 2018). The recent identification of *Fusarium moniliformis* as a pathogen affecting *M. dubia* expands the diversity within the *Fusarium* species complex. (Muthulakshmi and Pradipa 2025). The sudden mortality and decline of one year old plants in *M. dubia*-based agroforestry system at the ICAR–Central Agroforestry Research Institute, Jhansi, formed the basis for the present investigation. Despite growing knowledge, a critical gap remains in the precise characterization of root rot and wilt disease of *M. dubia*, as previous reports have largely attributed to the morphologically variable *Fusarium solani* species complex (FSSC) without species-level resolution. Consequently, accurate species-level identification of the pathogen is a prerequisite for developing effective disease management strategies, including the selection of resistant germplasm and the implementation of targeted nursery interventions. In the present study, the gap has been addressed by employing a polyphasic approach that integrates morphological characterization, pathogenicity assays, combined with multi locus sequence based phylogenetic analysis.

Materials and Methods

Studies on disease symptomatology and morphological characterization

M. dubia tree mortality was observed in 2021 at ICAR- Central Agroforestry Research Institute, Jhansi (25.503" N/ 78.542" E), that falls in semi-arid zone of central India. A critical record of the symptomatology of affected trees was maintained, and the infected plants were removed by digging the soil until the roots were exposed and carefully collected samples in sterile bags for further examination of vascular tissues, as well as for pathogen isolation. The vascular tissues of the infected roots were aseptically excised after thorough washing of adhered soil under running tap water, then cut into 1 mm-size pieces. Surface sterilization was carried out by 1.0% sodium hypochlorite and 70% ethanol, each for one minute duration. Then the segments were rinsed in sterile water three times sequentially and blot-dried on autoclaved filter paper, which were then aseptically placed onto PDA medium (HiMedia Laboratories, Mumbai, India). Radial growth of mycelium was observed after 48 hours, followed by subculturing, the fungus was purified by single-spore isolation method and identified with the help of the standard morphological and microscopic study (Pandey et al. 2018) after 7 days of incubation with alternate fourteen hours light and eight hours dark photoperiod.

Molecular characterization by Multi Locus Sequence Analysis

For molecular identification, pure culture of the MFs1 isolate was grown on Potato Dextrose Broth (HiMedia Laboratories, Mumbai, India) and incubated at 26°C for five days under continuous agitation at 120 rpm in an orbital shaker incubator. Mycelium was then aseptically harvested, and genomic DNA was extracted using the CTAB method (Doyle and Doyle 1987). DNA quality and concentration were assessed using a NanoDrop spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). PCR amplification was performed targeting four genetic loci: (i) the internal transcribed spacer (*ITS*) region using primers ITS1-'TCCGTAGGTGAACCTGCGG' and ITS4 - 'TCCTCCGCTTATTGATATGC' (White et al. 1990); (ii) Translation Elongation Factor 1-alpha (*TEF1*α); (iii) the largest subunit of RNA polymerase (*RPB1*); and (iv) the second largest subunit of RNA polymerase (*RPB2*). Primer sequences for *TEF1α* were EF595F-'CGTGACTTCATCAAGAACATG' and EF1160R- 'CCGATCTTGTAGACGTCCTG' as specified by White et al. (1990), while RPB1 and RPB2 primers followed O'Donnell et al. (2010): RPB1 [Fa: CAYAARGARTCYATGATGGGWC; G2R: GTCATYTGDGTDGCDGGYTCDCC] and RPB2 [5f2: GGGGWGAYCAGAAGAAGGC; 7cr: CCCATRGCTTGYTTRCCCAT]. Thermal cycling conditions, performed in an Eppendorf thermocycler (Hamburg, Germany), were based on protocols from O'Donnell et al. (1998, 2010) and included an initial denaturation at 94°C for 5 min, followed by 30 cycles of denaturation at 94°C for 1 min, annealing at 55°C for 1 min, and extension at 72°C for 1 min, with a final extension at 72°C for 5 min and a hold at 4°C.

PCR products were sequenced by Green genome Pvt. Ltd. Amplified fragments were visualized on 1.0% agarose gels, sequenced bidirectionally, and then curated and assembled using BioEdit software (Hall 1999). Multiple sequence alignments were performed using CLC Sequence Viewer 8.0 (Qiagen,

http://www.qiagenbioinformatics.com), with reference sequences retrieved from the NCBI database. The curated sequences were analyzed and compared against both the Fusarium-MLST (Multilocus Sequence Typing) database (https://www.fusarium.org/page/ID), which enables precise species-level identification within the genus *Fusarium* (O'Donnell et al. 2022), and GenBank via BLAST (Basic Local Alignment Search Tool). Annotated sequences were submitted to NCBI GenBank.

For phylogenetic analysis, concatenated sequences (3900 bp) from four loci (*ITS, TEF1α, RPB1* and *RPB2*) of 21 Fusarium species were assembled into a final dataset and a phylogenetic tree was constructed using MEGA 11 (version 11.0.13) employing the Maximum Likelihood method by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using Tamura Nei model with 1,000 bootstrap replications (Tamura and Nei 1993; Tamura et al. 2021). To determine the genetic relationships with other *Fusarium* species, members of the *Fusarium incarnatum–equiseti* species complex (FIESC) and *Fusarium oxysporum* species complex (FOSC) were designated as outgroups.

Pathogenicity assays

For the pathogenicity assay, 3 months old seedlings (n = 10) of *M. dubia* were planted and allowed to grow in sterile garden soil in 30 cm dia. plastic pots for 15 days. The representative isolate was multiplied on maize-sand meal (Pandey et al. 2018) and incubated at 27° C for 30 days. The inoculum was mixed in pot soil before planting the seedlings for challenge inoculation (Thao et al. 2021), and to ensure further infection 10 ml of spore suspension ($1x10^4$ conidia/ml) was poured at the collar region of each seedling without wounding them and observations were recorded on disease appearance. Seedlings (n = 10) treated with sterile distilled water served as control. All the plants were maintained under controlled glasshouse conditions, having 26°C ± 2 with a 12 h photoperiod and 90% Relative humidity (RH). The symptomatic plants were selected and the causal agent was re-isolated for the confirmation of the identity and pathogenicity of original isolates. The experiment was repeated for the confirmation of the results with similar number of replications. Finally, the isolate MFs1 was deposited in the National Centre for Microbial Resources (NCMR), Pune, Maharashtra, India.

Results

Etiological investigation on M. dubia root rot and wilt

During the year 2021, sudden and progressive wilt symptoms were observed in one year old *M. dubia* plantation at a research farm in the ICAR - Central Agroforestry Research Institute (28.6410" N: 77.1531" E), Jhansi, India. Careful observation of the above ground symptoms of infected trees (Fig. 1a) revealed yellowing, drying, and premature loss of foliage, discoloration and drying of the branches, stem (Figs. 1b), and death of the trees (Fig. 1a). After uprooting the infected trees, disintegration of bark around the collar region, rotting of roots (Figure c), and brownish discoloration of vascular tissues

(Figs. 1d) were observed without a foul smell. The severity of mortality of plants showing wilt incidence was 90 percent as observed in both the years even after replantation.

The infected root tissue of one-year old trees showed whitish cottony aerial mycelium with dark purple diffusing pigment at the center on PDA media that can covered 90 mm diameter within 5 days of incubation at 26^{O}C temperature (Fig. 1e). The pure culture was maintained by single spore isolation, and the isolate was named as MFs1. Further microscopy revealed hyaline, septate, and branched mycelium (Fig. 2a); distinct falciform (sickle-shaped) macro conidia, thick-walled measuring approximately 25–30 x 4–5 um, with predominantly 3 transverse septa, featuring a well-developed foot cell (basal cell) and a blunt apical cell with a gentle dorsiventral curvature (Fig. 2b). Microconidia are thin-walled, abundant, 0 or 1 septate, oval to reniform in shape (Fig. 2b). Chlamydospores are abundantly present in 15–30 days old culture, formed directly within the hyphae (intercalary) or terminal, singly or in pairs (Fig. 2c). Further, the strain was deposited in NCMR, Pune, India, as *Fusarium falciforme* MFs1 with accession no. MCC9877.

In pathogenicity assays infected plants showed leaf yellowing as an initial symptom on select branches of the lower stem at six days post-inoculation (dpi) (Fig. 3a). Subsequently, symptoms progressed to other parts of the plant, drooping of leaves, sectored wilting was observed in all inoculated plants by 12 dpi (Figure Bb). The entire plant showed signs of infection by 20 dpi, with affected leaves developing a brown, blighted appearance and ultimately exhibiting typical wilt symptoms, consistent with those observed under natural field conditions (Fig. 3c). Control plants treated with sterile water remained asymptomatic (Fig. 3d) even at 20 dpi. Brown discoloration was observed in artificially infected roots and vascular tissues, from which the pathogen was re-isolated using the previously described procedure. The re-isolated fungi displayed cultural and microscopic characteristics identical to the isolate MFs1. In contrast, no such infection was observed or fungi recovered from control seedlings. The experiment was repeated, and the re-isolated fungus was re-sequenced using ITS1/ITS4 primers, with its identity confirmed through the *Fusarium* MLST and NCBI databases. Collectively, these observations and datasets from the pathogenicity assay proved Koch's postulates.

Molecular identity and phylogenetic analysis

The molecular characterization based on Multi Locus Sequence analysis of isolate MFs1 using concatenated sequence (3900 nt) of four loci *ITS* – 569 bp, *TEF1α*- 681 bp, *RPB1–1607* bp, and *RPB2–1043* bp revealed closest sequence similarity to members of *Fusarium falciforme* (NRRL 32313–100%, NRRL 28562, NRRL 28563 – 99.92%), of the *Fusarium solani* species complex (FSSC) within the *Fusarium* MLST database. The assembled, curated and annotated sequences were deposited in GenBank under the following accession numbers: *ITS* (OM980195; 569 bp), *TEF1* (PZ269500; 681 bp), *RPB1* (PZ158280; 1607 bp), and *RPB2* (PZ269501; 1043 bp). Further, phylogenetic analysis using concatenated sequences involved nucleotide sequences from 51 *Fusarium* strains of 21 species with all four loci. There was a total of 6668 positions in the final data set and tree with highest log likelihood was shown based on

Maximum Likelihood method and Tamura-Nei model. The polyphasic phylogenetic analysis revealed close clustering of *Fusarium falciforme* MFs1 with other *Fusarium falciforme* strains in FSSC clade and was distant from members of *Fusarium incarnatum* (FIESC) and *Fusarium oxysporum* (FOSC) clades (Fig. 4). This confirmed the identity of *M. dubia* root rot and wilt causing strain MFs1 as '*Fusarium falciforme*'.

Discussion

The global demand for timber is growing by 1.7% annually (South, 1999) and *M. dubia* is taking momentum to meet the current demand through commercial agroforestry and social/community forestry with its unique growth and wood quality properties. India, West Java and Australia have most of the agroforestry under *M. dubia* cultivation (Rambey et al. 2019). However, the recent decline of *M. dubia* is of great concern due to its susceptibility to vascular wilt and root rot disease. In 2021, a sudden outbreak in wilt and root rot disease that led to mortality of one year old *M. dubia* trees was observed in ICAR – CAFRI, Jhansi, India. Infected trees showed root rot, wilting, yellowing, premature senescence and loss of foliage, irreversible drying of branches and whole tree mortality with 90% severity. Previous reports suggested that wilt disease caused by *Fusarium solani* damages *M. dubia* from the nursery to the mainland, causing heavy losses even in established plantations (Muthu Kumar et al. 2014; Pandey et al. 2017). Vascular wilts, are in, general caused by several soil- inhabiting *Fusarium* species in various tree species. *Fusarium xylarioides*, known to cause Coffee Wilt Disease (CWD), is endemic in several African countries, affecting commercially important coffee species and causing serious economic losses (Musoli et al. 2008). Banana Fusarium Wilt (also known as Panama disease) is one of the most disastrous plant diseases incited by *Fusarium oxysporum f. sp. cubense* race 4 (FOC4). Recently, *Fusarium kalimantanense* was also reported from Brazil causing banana wilt (Santos et al. 2022). Cashew (*Anacardium occidentale* L.) is the leading economical tree crop in Tanzania, generating income to over 2.5 million farmers. Despite its importance, cashew is threatened by a new serious destructive disease called Cashew Fusarium Wilt (CFWD), which is caused by *Fusarium oxysporum* (Mbasa et al. 2021). Similarly, several other tree species including shisham and apple (*Fusarium solani*), tung tree (*Fusarium oxysporum* f. *sp. fordiis* (*Fof*-1)), guava and citrus (*Fusarium oxysporum* Sch.) are also susceptible to Fusarium wilt (Bakshi, 1954; Yan et al. 2018; Cao et al. 2021; Hussain et al. 2012, Hannachi et al. 2014). In our study, the *M. dubia* wilt and root rot pathogen was isolated and identified based on poly phasic approach, involving cultural, morphological characters, pathogenicity, and multi locus sequence analysis. Morphological traits, including falcate macroconidia, colony pigmentation, and growth patterns on PDA media, correspond closely with established descriptions of *F. falciforme* and align with previous descriptions of the species *F. falciforme* documented by various researchers (Leslie and Summerell, 2006; Gangaraj et al. 2022; Thao et al. 2021; Pandian et al. 2022; Xu et al. 2023; Madhavan et al. 2025). The pathogenicity assay replicated field symptoms under controlled conditions, fulfilling Koch's postulates and confirming the virulence of the isolate MFs1. In general, Multi locus sequence analysis of four genes (*ITS, TEFf-1α, RPB1*, and *RPB2*) is recommended to distinguish between Fusarium species (O'Donnell et al. 2010; 2013; Crous et al. 2021). In present study, the molecular

phylogenetic analyses based on concatenated sequences of *ITS, TEFf-1α, RPB1*, and *RPB2* distinctly separated the MFs1 from other FSSC members including *Fusarium solani* and related *Fusarium* species complexes, reinforcing the taxonomic resolution as '*F. falciforme*' (O'Donnell et al. 2010; Crous et al. 2021).

The identification of *F. falciforme* as *M. dubia* wilt and root rot pathogen, expands the known host range, which has been reported on many agricultural hosts across Asia, the Americas, Africa, and European production systems. It causes wilt and root rot in strawberry, tomato, melon, papaya, chickpea, bean, soybean, cowpea, cassava, alfalfa, wheat, barley and also infects ornamentals, including *Chrysanthemum*, *Epimedium sagittatum*, *Weigela florida* and *Spathiphyllum wallisii* (Payán et al. 2024; Madhavan et al. 2025). This study underscores the adaptive potential and phytosanitary threat posed by *Fusarium* species in agroforestry systems. Given the ecological and economic importance of *M. dubia* as a fast-growing timber species with high biomass yield (Rambey et al. 2019; Saravanan et al. 2014), the emergence of *F. falciforme* as a dominant wilt pathogen represents a significant challenge to sustainable cultivation. In an effort to manage the disease in *M. dubia*, soil drenching with carbendazim (1g/litre) deployed as a management strategy, but only 15% survival was achieved. Previous reports suggest that potted banana plants that were inoculated with an endophyte from rubber tree *Serratia marcescens* ITBB B5-1 before infection with *Fusarium oxysporum f. sp. cubense* race 4 (FOC4) showed 78.7% reduction in the disease severity index in the greenhouse experiments. In the field trials, ITBB B5-1 showed approximately 70% inhibition against the disease, indicating the biological control potential of Banana Fusarium wilt (Tan et al. 2015). Taken together, in the present scenario, investigating the feasibility of controlling *Fusarium* wilt and root rot in *M. dubia* with natural bioinoculants is considered as one of the strategic steps towards sustainable management.

Declarations

Conflict of interest statement

The authors declare there is no conflict of interest in the submission of this manuscript

Ethical statement

Our manuscript entitled "**First Report ofFusarium falciformeinciting Wilt and Root Rot in Malabar neem (Melia dubia) by Polyphasic Characterization from the Bundelkhand Region of Central India"** complies with the ethical rules applicable to the journal 'Archives of Microbiology'.

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Author Contribution

M.A -Conceptualization, Methodology, Validation, Data analysis, and Manuscript writing. M.A, V.Y.N, A.Y, S.B, A.B -Carried out the plant analysis, Molecular studies, Manuscript preparation and Data analysis. M.A, K.R, A.K, A.K.H- Manuscript preparation. All authors read and approved the final manuscript.

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Data Availability

The gene sequences were deposited to NCBI (https:// www.ncbi.nlm.nih.gov/genbank), and the accession numbers were mentioned in the manuscript.

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Figures



Figure 1

Symptomatology and field diagnosis of wilt and root rot disease caused by *Fusarium falciforme* in *Melia dubia*

a) Infected that succumb to disease in *Melia dubia* trees based agroforestry system showing yellowing, premature senescence, loss of foliage, barren branches, drying of the whole tree and mortality; b)

Discoloration and drying of branches; c) Bark disintegration at collar region, rotting of the roots without foul smell; d) Brownish discoloration of internal vascular tissues; e) Pinkish white aerial mycelium with dark purple diffusing pigment on potato dextrose agar media

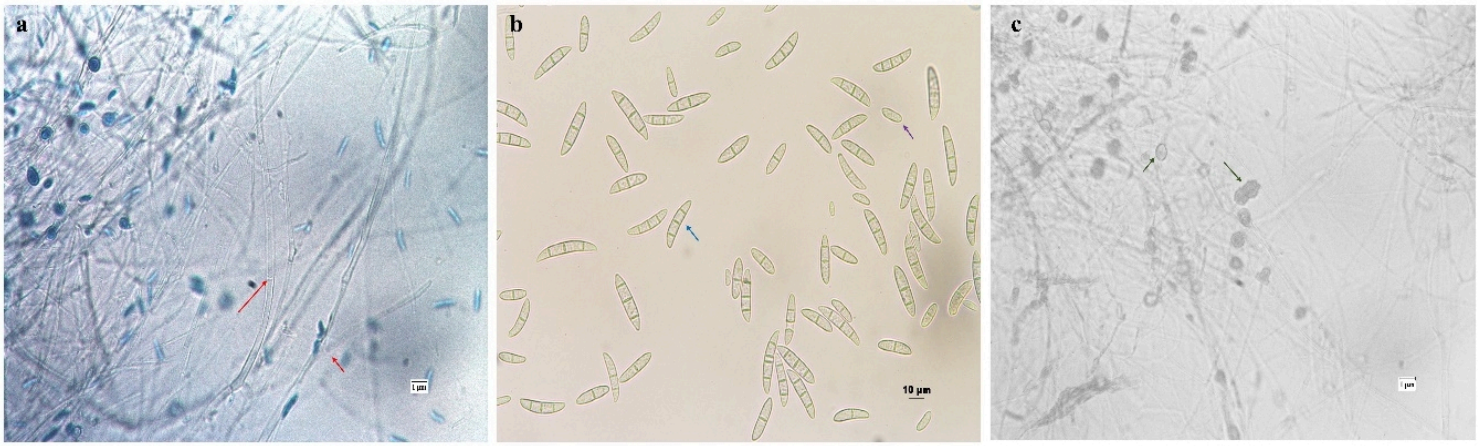


Figure 2

Microscopic mycelial and conidial characters of *Fusarium falciforme* MFs1

a) Hyaline, septate and branched (red arrows) mycelium; b) Macroconidia with 3 to 4 septa, cylindrical, thick-walled, moderately curved falcate, with a blunt and round apical cell and distinctly foot-shaped or notched basal cell (blue arrow); thin-walled, 0 or 1 septate, oval to reniform in shape micro conidia (purple arrow); c) chlamydospores formed directly within the hyphae (intercalary) or terminal, singly or in pairs (green arrows)

Note: Microscopy was performed using Nikon E400 compound microscope at 40x magnification



Figure 3

Pathogenicity assay by artificial inoculation of *Fusarium falciforme MFs1* on *Melia dubia* seedlings

a) Infected plants showed yellowing of lower leaves as an early symptom at six days post inoculation (dpi); **b**) Yellowing advanced to other parts of the seedling, drooping of leaves, consequently causing sectored wilting of the plant at 12 dpi.; **c**) Severely infected seedlings showed blightened appearance

with drying of leaves, branches, toppled down and died at 20 dpi; **d**) Plants treated with sterile water remained healthy even after 20 dpi served as control

Note: For pathogenicity assay, 3 months old seedlings (n=10) of Malabar neem, planted and allowed to grow in sterile garden soil in 30 cm dia. plastic pots for 15 days. The representative isolate was multiplied on maize-sand meal and the inoculum was mixed in pot soil for challenge inoculation (Thao et al. 2021), and to ensure further infection, 10 ml of spore suspension ($1x10^4$ conidia/ml) was poured at the collar region of each seedling without wounding them and observations were recorded on disease appearance. Seedlings (n=10) treated with sterile distilled water served as control. All the plants were maintained under controlled glasshouse conditions having 26° C ± 2 with 12 h photoperiod and 90 % Relative humidity (RH).; **dpi** – days post-inoculation.

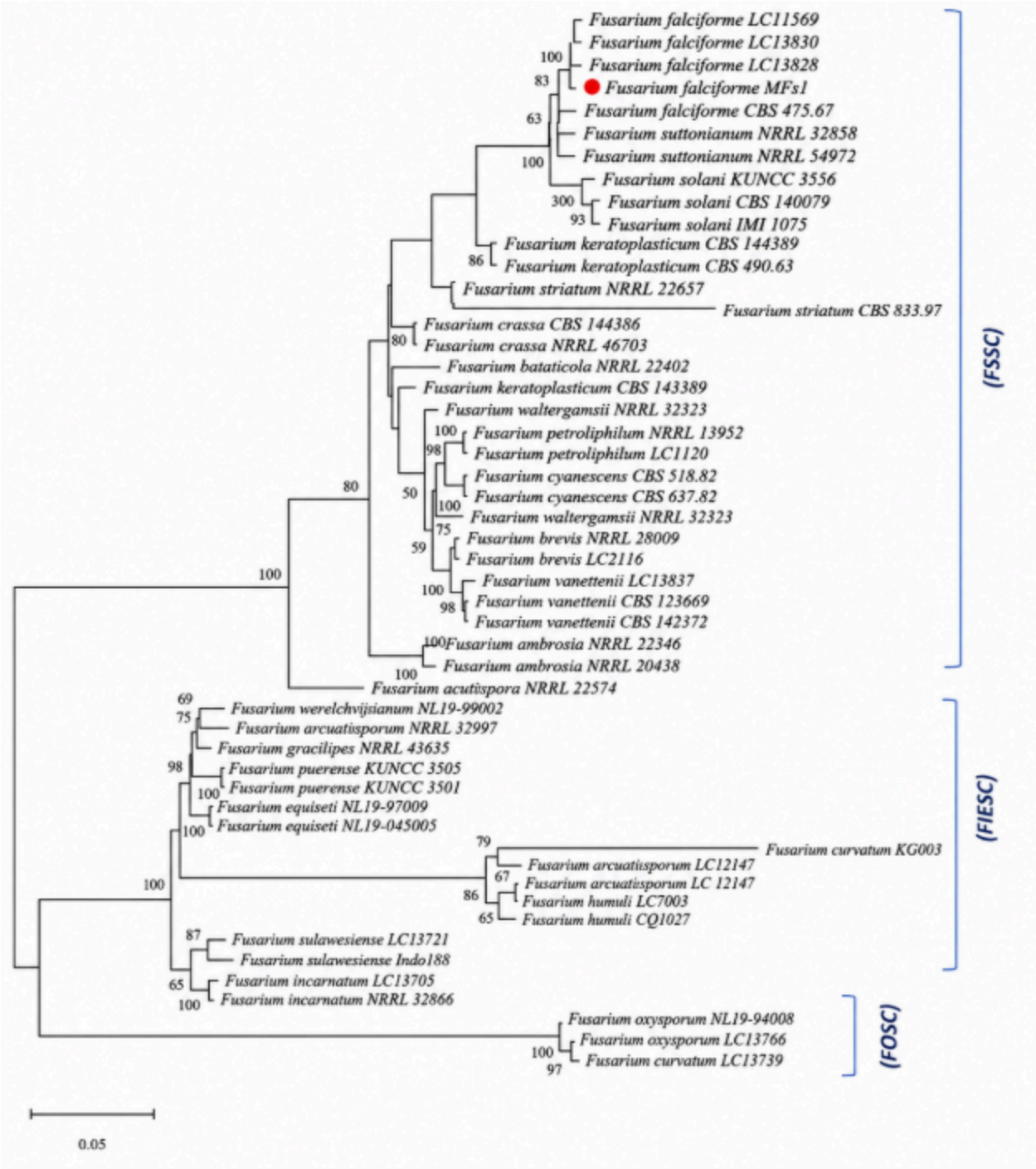


Figure 4

Phylogenetic analysis of *Fusarium falciforme MFs1*from wilt and root rot infected *Melia dubia*

Phylogenetic analysis was performed using concatenated sequences of *ITS*, *TEF1α*, *RPB1* and *RPB2*genes obtained from 21 species of *Fusarium* strains by the Maximum-Likelihood method. *Fusarium falciforme*MFs1 shared the same clade (red circle) with reference sequences of *Fusarium falciforme* isolates (FSSC) and distant from other members of FSSC clade, whereas *Fusarium incarnatum* (FIESC) and *Fusarium oxysporum* (FOSC) formed separate clades. This analysis involved 51 nucleotide sequences consisting of all four genes and there was a total of 6668 positions in the final dataset.

Evolutionary analyses were conducted in MEGA11 (Tamura et al. 2021), by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using Tamura-Nei model (1993); (**FSSC)** - *Fusarium solani* species complex; (**FIESC)** - *Fusarium incarnatum-equiseti* species complex; (**FOSC)** - *Fusarium oxysporum* species complex