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Exploring Foliar and Soil Pathogens Impacting *Melia dubia*: Documentation and Characterization

P. Muthulakshmi[1], C. Pradipa[*2] and S. Kavitha[3]

[1]*Department of Plant Pathology, Tamil Nadu Agricultural University, Coimbatore, India*
[2]*Department of Agro Climate Research Centre, Tamil Nadu Agricultural University, Coimbatore, India*
[3]*Department of Seed Science, Tamil Nadu Agricultural University, Coimbatore, India*

ABSTRACT

Melia dubia, is a species known for its fast growth and available market, but it has certain challenge associated with pathogens infecting soil and foliage. Identification of the diseases and studying the cultural and morphological characteristics of foliar and soil-borne pathogens associated with Melia dubia can provide valuable insights into their identification, behavior, and management. The identification of multiple pathogens associated with foliar and soil-borne diseases, requires a thorough understanding of their diverse growth patterns, cultural behaviors, and morphological traits. The purpose of this study is to focus on the major foliar diseases and soil-borne diseases. The study involved collection of the diseased samples from the nursery and plantation. Later, isolation of pathogens from infected Melia dubia seedlings and trees using Potato Dextrose Agar (PDA) and identifying them based on cultural and morphological characteristics. Key pathogens identified include Fusarium moniliformis, Phoma sp, Rhizoctonia solani , Lasiodiplodia theobromae , and Pythium sp. The research outlines their growth on different media and provides detailed observations on colony and conidial characteristics. This comprehensive analysis underlines the need for targeted research and management strategies to address disease outbreaks in Melia dubia plantations, emphasizing the importance of disease characterization for effective control measures.

Keywords: *Melia dubia, soil and foliar pathogen, cultural characterization, Morphological characterization, Fusarium, Rhizoctonia, Pythium, Lasiodiplodia*

1. INTRODUCTION

Melia dubia is an important tree species grown worldwide for its medicinal and timber values. It is widely used in the timber and pulp industry and also as an organic pesticide, fertiliser, agro-forestry and herbal formulation. It has rightfully earned its reputation as a "money-spinning tree" of short rotation due to the multitude of benefits it offers across various sectors. The intensive expansion of *Melia dubia* plantations indeed brings with it certain challenges, notably the increased incidence of diseases. The presence of diseases affecting *Melia dubia* at various stages of development, from nursery to harvest, underscores the importance of disease management throughout the tree's lifecycle. The presence of collar rot, root rot, seedling web blight, leaf spot and other diseases caused by fungal pathogens such as *Phoma sp*., *Fusarium moniliformis*, *Rhizoctonia solani* , *Colletotrichum dematium, Fusarium oxysporum, Armillaria fumosa, Cylindrocladium ilicicola, Lasiodiplodia theobromae*, and *Pythium sp*., as well as issues like nutrient deficiencies, drought, and insect pest pressures, lead to substantial losses in both nurseries and main fields.

Rhizoctonia solani is recognized as a significant pathogen causing collar rot and seedling web blight diseases in the nursery stage of *Melia dubia*.

Corresponding Author:* ***C. Pradipa*

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This fungus is known to be a significant forest nursery pathogen in Kerala, and it presents additional challenges due to its occurrence in different Anastomosis Groups (AGs) with varying levels of virulence [1]. It's noteworthy that both soil-borne and aerial strains of *Rhizoctonia solani* can cause web blight. The severity of web blight caused by *Rhizoctonia solani* is influenced by various nursery conditions, including humidity, temperature, and sanitation practices. Leaf spot caused by *Colletotrichum dematium* and *Cylindrocladium ilicicola* are additional diseases recorded on *Melia dubia* in nursery conditions.

Understanding the characteristics and interactions of these pathogens are essential for developing effective disease management strategies in *Melia dubia*. The lack of established etiology and characterization of diseases affecting *Melia dubia* in Tamil Nadu underscores the need for comprehensive research efforts in this area. This study takes a closer look at two diseases, collar rot and seedling web blight, caused by *Rhizoctonia solani* , which pose significant challenges, particularly in the seedbed nursery.

2. MATERIALS AND METHODS

2.1 Collection of diseased samples

An extensive field study was carried out in the plantations and nurseries located in and around Coimbatore district of Tamil Nadu and Forest College & Research Institute (FC & RI), TNAU, Mettupalayam, Tamil Nadu. Five plantations are owned by the farmers and the nursery as well as the plantations maintained by FC & RI, Mettupalayam. The samples were collected in a paper bag and transported to the laboratories at FC & RI, Mettupalayam for isolation and culturing.

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2.2. Isolation of the pathogen

For isolating the causative organisms from diseased parts of *Melia dubia* seedlings and trees, the samples were collected from FC & RI nurseries, TNAU, Mettupalayam and five plantations in farmer's fields using standard protocols. The samples are placed in clean polythene bags to prevent contamination and maintained sample integrity during transportation to the laboratory. The isolation of the causative organism is carried out promptly to avoid any saprophytic growth on the specimens. The tissue segment method, as described by [2], was employed for fungal isolation. The Potato Dextrose Agar medium was prepared according to the standard formulations and autoclaved to sterilize the medium and eliminate any potential contaminants. Autoclaving is typically performed at 121°C and 15 psi for 15 minutes to ensure complete sterilization.

The infected parts collected from the field are surface sterilized using 1% sodium hypochlorite solution for one minute. The surface-sterilized infected parts are then transferred onto petri plates containing Potato Dextrose Agar medium. The inoculated plates are placed in an incubation chamber at 25 ± 10°C to promote fungal growth.

2.3. Pure culturing and culture maintenance

Either single hyphal tip or single spore isolation method, as suggested by [3] was used for obtaining pure cultures of the fungus. Potato Dextrose Agar (PDA) slants were used to culture the single hyphal tip from the tissue segments. A sterile inoculation needle was used and the culture was maintained under aseptic conditions. In case of the single spore isolation method, serial dilution technique was used to prepare the diluted spore solution (8 to 10 spores/ml). The one ml of solution prepared was used to spread uniformly on a 2% water agar plates, any excess suspension was discarded using aseptic drainage. The cultures were maintained at 28±2 °C and observed continuously. The single spore germination was observed under light microscope and further cultured on PDA slants. These slants are incubated at 28±2 °C for 10 days. The mycelial bits obtained from the slants were cultured on the centre of the PDA medium containing petriplates and incubated for 10 days under room temperature. Sub culturing was developed in the PDA slants by using the culture developed in the petriplates, and preserved at 4 °C for further studies.

2.4. Characterization of pathogen

The cultural characteristics of various pathogens isolated on Potato Dextrose Agar (PDA) were examined in detail based on the colony colour, shape, texture, fruiting body formation, growth rate, and sporulation [4].

2.5. Morphological characteristics of the pathogen

Based on the cultural and morphological characteristics the identification of the pathogens was done at the species level. The culture of the isolated pathogen grown the petriplates was used on the glass slides for examining under the PC-controlled image analyzer at 100x magnification. The spore and mycelial characteristics were scrutinized under various microscopic fields, which were recorded. The conidial characters were studied by brushing the conidia using flooding plate technique.

The 7 days old cultures in the petriplates were used for preparation of aqueous suspension of conidia. Sterile gauze was used to extract/filter the conidia from the suspension. An image analyzer with specific software was employed for measuring the size of the conidia and chlamydospores, and to confirm the pathogen species standard references were used.

2.6. Culturing of the isolated pathogen on different solid medium:

The comparative study of the fungal growth in three different solid media was done in Completely Randomized Design (CRD) with three replications. The fungal mycelial growth of *L. theobromae*, *Pythium sp*p., *R. solani*, *Phoma sp*p., and *F. moniliformis* on Potato Dextrose Agar (PDA), Oat Meal Agar (OMA), and Malt Extract Agar (MEA) were compared. The inoculum for culturing the colonies was done by cutting out 5 mm culture discs from the young actively growing margins of the colonies of the pathogens. The inoculated media were maintained at 25±2°C for seven days. The results were statistically analyzed [5].

2.7. Culturing of the isolated pathogen in different liquid medium

As in case of comparative study of the fungal growth in three different solid media, the study was also done in three liquid media. The autoclaved media in 100 ml conical flasks were used to culture the pathogen. The actively growing mycelial discs of 7 mm diameter were cut and inoculated in the three different media separately and incubated for 8 days at ambient condition. A sterile muslin cloth was used to filter the mycelium from the grown culture which was weighed as wet and the dry weight was measured after drying in hot air oven at 60 °C for five days [6].

3. RESULTS

3.1. Isolation of the pathogens

The isolation process resulted in the identification of three different foliar fungal pathogens namely *R. solani* , *F. moniliformis* and *Phoma sp*. *R. solani* was isolated except FC & RI nursery and Farmers plantation; *F. moniliformis* was isolated from the farmer plantations, nursery and plantation of FC and RI, Mettupalayam; while *Phoma sp*. was isolated only from the samples collected from the FC and RI nursery

The samples with root rot and collar rot symptoms collected from the plantations in various locations resulted in *L. theobromae* and *Pythium sp. Pythium sp* was isolated from the samples collected from TNPL and Eden nursery whereas *L. theobromae* was found in samples collected from all the locations.

The Pure culture and sub culture of the isolated pathogens were developed and identified to species level based on the cultural and morphological characterization of the isolates.

3.2. Cultural characters of isolated pathogens

The cultural characteristics of the isolated pathogens such as the pigmentation, shape and growth pattern of the colonies were studied using Potato dextrose agar medium and the results are represented in the table 1 and the pictorial representation is given in Fig 3.

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Table 1. Cultural characteristics of the isolated pathogens associated with diseases of M. dubia

Pathogen	Growth rate of Mycelium	Colony characters
F.moniliformis	Full plate mycelial growth- within nine days	Initially the growth of colony was white in colour. Margin was smooth in texture. Topography was medium fluffy. The reverse side of the colony was light brown in colour.
Phoma sp	Full plate mycelial growth- within nine days	Colour of growth is characterized with pale green-olive, flocky aerial hyphae of the air mycelium with white edge. The reverse colony was olive grey.
R. solani	Full plate growth in Petriplate within seven days	The growth of colony was white in colour. Initially the mycelium aggregated and form ball like structure later it changed to brown colour sclerotia. The reverse sides were brown in colour.
L. theobromae	Full growth within five days	Initially the growth was feeble and white in colour and later changed to greyish black The colony showed a fluffy aerial growth. Aggregation of grey coloured mycelium and production of pycnidia in the centre of the colony were observed in eight days after inoculation. The reverse side of the colony was black.
Pythium sp.	Full growth within six days	Initially the colony was hyaline and later turned light brown in colour. Reverse side was brown in colour.

Figure 3. Cultural charactersitics of the isolated pathogens associated with M. dubia

3.3. Morphological characters of the isolated pathogens

The morphological characters of different pathogens isolated from the diseased samples collected from the *M. dubia* nursery and plantations are given below and depicted in the Fig. 4.

3.3.1. *F. moniliformis*

F. moniliformis was identified based on the macro and microconidial characteristics. The shape of the macroconidia was long, slender with a length of 19.631 µm to 20.624 µm and breadth of 3.578 µm. The elongated or whip like apical cells were found and it were tapered. The size of the microconidia was 6.648 µm to 7.250 µm length and 2.600 µm to 4.079 µm breadth.

These characters of macroconidia, micro conidia and apical cells are the distinguished characters of *F. moniliformis*.

3.3.2. *Phoma sp*

The presence of black coloured Pycnidia and presence of spores ,concentric circles within the pycnidia supported the identification of the pathogen as *Phoma sp*. The size of the pycnidia was 68.9 µm x103.6 µm .

3.3.3. *R. solani*

The septated, perpendicularly branching hyphae with constrictions at the point of branching of the hyphae, along with the eminent feature of presence of septa near the branching junction are the features of the fungi *R. solani* .

3.3.4. *L. theobromae*

Unicellular, ellipsoidal shaped, thick walled hyaline conidia with granular content which on maturity turned dark brown with longitudinal striations is the key feature of *L. theobromae* and with the length of 20.54 µm and breadth of 10.47 µm supported the identification.

3.3.5. *Pythium sp*

The presence of Oospores and Sporangia are the features of *Pythium sp*.. The dimensions of sporangium were also found to be 7.181 µm which added on to the evidence.

F. moniliformis (Conidia)

F. moniliformis (hyphae)

Phoma sp. (Conidia)

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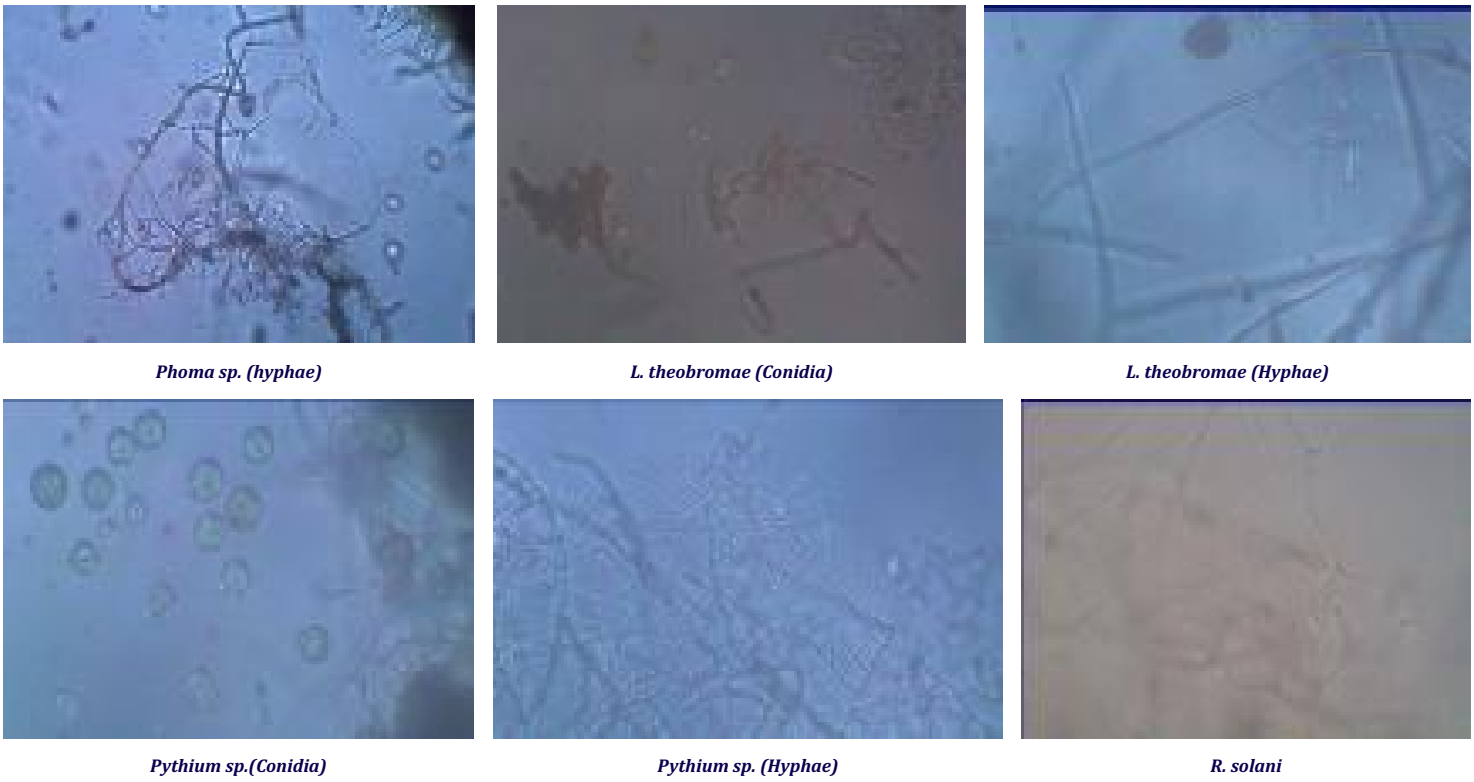

Figure 4. Morphological characteristics of the isolated pathogens associated with M. dubia.

4 DISCUSSION

4.1. Isolation of the pathogen

The leaf samples of *M. dubia* that were collected with the symptoms of leaf spot and leaf blight were used for the isolation of the pathogens in PDA and were identified as *Fusarium moniliformis*, *Rhizoctonia solani* and *Phoma sp*.

F. moniliformis was reported to be an important pathogen of forest seedlings in nursery [7], isolated from the stalks of Maize and Sorghum [8] and also an important contributor for damping-off complex in Pine sp. [9].

R. solani was found to be the dominating pathogen in the forest nurseries of Shimoga District [10], also isolated and identified from leaves of *Tectona grandis, Khaya senegalensis* and *Polyalthia longifolia*, [11].

Similarly, *Phoma sp*. was detected to cause leaf spot in *Aegle marmelos, Cassia fistula, Tectona grandis* and *Madhuca latifolia* [12], Olive trees [13], *Archangelica officinalis* [14], *Salvia nemorosa* [15], *Elaeis guineensis* [16] and Oregano [17].

Whereas from the root samples collected with root and collar rot symptoms L. theobromae and *Pythium sp* were spotted to be the causal agent. Similarly, *Lasiodiplodia theobromae* was identified and isolated from root rot samples of mulberry [18] and Cocoa [19].

[20] characterized and isolated *Pythium* from Olive trees with symptoms of root rot and [21] reported that the pathogen causing root rot, damping off and wilting in Acacia was *Pythium sp*.

4.2. Characterization of *F. moniliformis*

The colony of *F. moniliformis*, which was found to cause leaf spots was initially white with smooth margin and was moderately fluffy. The underside of the colony was brown. Analysis of the morphological characteristics revealed the long slender macro conidia with elongated or even whip-like and tapered apical cells. The macro conidia were characterized by prominent and 19.63 to 20.62 µm long basal cells. The microconidia was also found, which were elongated and foot-shaped, ranged in length from 6.65 to 7.25 µm.

[22] characterized *F. moniliformis* with white mycelium, hyaline macro conidia that are narrow and straight, and abundant, hyaline, oval-to clavate single-celled micro conidia. Similarly, [23] characterized *F. moniliformis* to be white, cotton candy and Pearl coloured colony with curved, tapered to a point, notched or foot shaped micro conidia of length ranging from 4.00-34.60 µm x 2.10-3.30 µm with no septa and macro conidia ranging from 37.10- 48.60 x 4.00-4.10 µm.

4.3. Characterization of *R. solani*

The isolates from the infected leaves grown on PDA medium initially aggregated and exhibited white mycelium which later developed into a ball like structure with brown sclerotia. The underside was also brown. The hyphae were hyaline, septated and branched at right angles. There were constrictions and a septum near the branching junction. All the samples resulted in colonies with similar characters. Similar kind of findings was reported by [24]. The unique character of branching of hyphae at right angles and a septum near the branching were reported by many authors [25,26].

4.4. Characterization of *Phoma sp.*

The colonies with pale olive green colouration, septate, branched grey mycelium and flocky aerial hyphae with edges in white colour. The underside colour of the colonies was olive grey. The colonies were characterized by black pycnidia that were 68.91 µm long and 103.61 µm wide. The spores were arranged in concentric circles. The colonies were described to be olivaceous and woolly with dark brown to black pycnidia [27].

4.5. Characterization of *L. theobromae*

The cushiony colonies of the isolates were greyish black and were characterized with black coloured pycnidia. These characteristics of the colonies were in agreement with that of [28]. Black mature and hyaline immature conidia were found.

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From the black pycnidia, black mature conidia of length varying from 15 to 24 µm and width of 7-12 µm were observed. Similar kind of conidial characteristics were also reported by [29].

4.6. Characterization of *Pythium*

The matured colonies were hyaline and later turned light brown. The underside of the colonies was observed to be brown coloured. Numerous oospores like bodies with hyaline and granular cytoplasm were also recorded. The sporangiopores were 7.18 µm. All the isolates from different locations showed similar characteristics. Similar morphological and cultural charateristics of *Pythium sp*. were reported by [30].

5. CONCLUSION

The present study on documentation of foliar and soil-borne pathogens affecting *Melia dubia* and observation of cultural and morphological characteristics added valuable insights into their identification, etiology and development of management. The important pathogens identified include *Fusarium moniliformis*, *Phoma sp*, *Rhizoctonia solani*, *Lasiodiplodia theobromae* , and *Pythium sp*. The research on cultural and morphological characterization provides detailed observations on colony and conidial characteristics of the pathogens. This study emphasizes the need for targeted research and management strategies to address disease outbreaks in *Melia dubia* plantations in future.

FUTURE SCOPE OF THE STUDY

The future scope of studying Melia dubia and its pathogens encompasses several key areas. It includes using molecular tools for precise pathogen identification and exploring pathogen diversity, investigating the biochemical interactions and environmental factors such as temperature, humidity, and soil conditions, and developing integrated disease management strategies. Additionally, breeding Melia dubia for resistance to specific pathogens could enhance plantation sustainability. The impact of climate change on pathogen behavior and plant vulnerability should also be explored. Establishing long-term monitoring programs will ensure that emerging diseases are tracked and managed effectively, leading to more resilient and productive plantations.

CONFLICT OF INTEREST

The authors report there are no competing interests to declare.

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