Original Research Article

Studies on Morphological Variability of the Fungal Pathogen,
Lasiodiplodia theobromae causing Dieback in Mango

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Abstract

Mango (Mangifera indica Linn.) is the “king of fruits” originated from South East Asia. Mango is affected by many biotic stresses; among them dieback is one of the serious diseases incited by the fungus Lasiodiplodia theobromae. Roving survey conducted in Tamil Nadu revealed the maximum disease incidence of 55.84% in Mulaiyur village (ILtDM10) of Dindigul district and minimum disease incidence of 8.95% in Ettipatti village (ILtDM17) of Krishnagiri district, Tamil Nadu. Major symptoms observed were defoliation of infected leaves, tip dieback, bark, twigs drying, shrivelling of twigs, vascular discoulouration, and finally death of plants. Thirty numbers of pathogen isolates were collected from the diseased plant parts collected during the survey and pure cultures of them were established. The morphological characteristics of the pure cultures were studied and the pathogen growth was initially white, later on grey to dark greyish black with black to dark black pigmentation. Conidia were initially hyaline, unicellular, sub ovoid to ellipsoid and mature conidia were dark brown to black, bi-celled, ellipsoid, thick walled with longitudinal striations. The size of the conidia varied between 18.4 - 26.8 × 10.6 - 14.2 µm.

Keywords
Mango dieback, Lasiodiplodia theobromae, Morphological variability

Introduction

Mango (Mangifera indica Linn.) “King of fruits” has its origin from South East Asia. It belongs to the family Anacardiaceae. It is a major fruit tree grown in more than 90 countries in tropical and subtropical regions of the world (Al-Jabri et al., 2017). India rank first among the mango producing countries accounting for 42% of the world production, followed by China 11% (FAO, 2015). In India, area and production is about 2.313 million ha and 22.353 million tonnes respectively (NHB, 2018 - 2019). The major mango growing states in India are Uttar Pradesh, Tamil Nadu, Andhra Pradesh, Bihar, Karnataka and Gujarat.
Mango is affected by various biotic and abiotic stresses that reduce the quantity and quality of the produce. The important diseases viz., anthracnose, dieback, powdery mildew, and mango malformation are the major constraints in mango production in India. Among these, the most destructive disease is dieback incited by the fungus *Lasiodiplodia theobromae*. It causes yield loss in field (pre-harvest disease—dieback) as well as in storage condition (post-harvest diseases—Stem end rot/fruit rot). This disease has been reported in several countries viz., India, China, Pakistan, Brazil, USA, UAE, Korea, Oman (Sharma *et al.*, 1994; Ploetz *et al.*, 1996; Al-Adawi *et al.*, 2003; Khanzada *et al.*, 2004; de Oliveira Costa *et al.*, 2010; Hong *et al.*, 2012; Saeed *et al.*, 2017).

This pathogen is a hemibiotrophic plant pathogen (Tudzynski and Sharon, 2003) and causes severe damage to its hosts. It causes variety of symptoms and named based on the affected plant parts and symptoms such as dieback, gummosis, stem end rot, blights, stem necrosis, root rot, leaf spot etc., (Punithalingam, 1980; Úrbez-Torres *et al.*, 2008). The symptoms are primarily observed at twigs, subsequently spreading to its branches followed by infection in all branches, ultimately resulting in death of the plant. The present research focuses on survey, pathogen isolation and studying the morphological variability in the pathogen causing mango die-back.

**Materials and Methods**

**Survey and collection of diseased samples**

Roving survey was taken up in various mango growing hotspots in Tamil Nadu during summer, 2019. The percent dieback incidence was assessed in 30 villages representing 10 districts (Table 1). Data like location (latitude, longitude), variety, age of trees and disease incidence were collected. Disease incidence (%) was calculated by the following formula (Teng and James, 2002).

\[ I(\%) = \left( \frac{ni}{N} \right) \times 100 \]

where \( I = \) Disease incidence (%), \( ni = \) total number of diseased trees, \( N = \) total number of trees observed.

Variation in the symptoms in mango plants caused by the disease was also recorded.

**Isolation and identification of the pathogen**

During the survey, infected plant samples (twigs) were collected and used for isolation of pathogen. The collected twigs were approximately cut into 0.2 – 0.5 cm in size (Saeed *et al.*, 2017) and surface sterilised using 1% sodium hypochlorite for 2 min followed by gentle rinse in sterilized distilled water three times and tissues dried using sterilized tissue paper (Al-Jabri *et al.*, 2017). The surface sterilized tissues were placed on Petri dish containing sterilized Potato Dextrose Agar (PDA) medium amended with the bacterial antibiotic, streptomycin sulphate and incubated at room temperature for 3 days. The pure culture of the pathogen was obtained by single hyphal tip method (Dhingra and Sinclair, 1985) and stored in PDA slants at 4°C for further studies.

**Morphological variability**

Morphological variability of pathogenic isolates was studied by growing the purified isolates on PDA medium. Cultural characters viz., colour (observe and reverse), topography, margin, zonation and days taken to cover the Petri dish were recorded (Sathya *et al.*, 2017). Spore characters were also studied based on the production of pycnidium and spores, colour, shape and size (length and breadth) of the spores using compound microscope.
(Phillips et al., 2013). The intensity of sporulation was measured by using the following grades: - no, + poor, ++ medium, and +++ good (Sathya et al., 2017).

**Results and Discussion**

**Survey and collection of diseased samples**

Roving survey on disease incidence in 30 different locations revealed the extent of dieback disease infection in major mango areas of Tamil Nadu (Table 1). Among thirty villages, maximum disease incidence was recorded in Mulaiyur village (ILtDM10) in Dindigul district (55.84%) followed by Andiyur village (ILtDM19) in Krishnagiri District with 53.84% disease incidence. The minimum disease incidence was recorded in Ettipatti village (ILtDM17) of Krishnagiri District (8.95%) (Plate 1; Fig. 1). Similarly, dieback disease incidence was reported to be 30 – 40% in Uttar Pradesh (Prakash and Srivastava, 1987), 0 – 40% in Andhra Pradesh (Madduleti, 1989), 89.4% in Al Batinah region of Oman (Al Adawi et al., 2006), 3.71 – 29.71% in Peru (Rodríguez-Gálvez et al., 2017), 6 – 42% in Oman (Al-Jabri et al., 2017).

**Table.1 Survey for incidence of mango dieback disease in Tamil Nadu**

| S. No. | Latitude | Longitude | Village | District | Isolate Code | Variety | Age of trees (Years) | Disease Incidence (%) |
|--------|----------|-----------|---------|----------|--------------|---------|---------------------|-----------------------|
| 1      | 29.7502  | 78.206805 | AC&RI (MDU) | Madurai | ILtDM1 | Bangalore | 10 | 12.90 |
| 2      | 29.94748 | 78.974122 | Melakkel | ILtDM2 | Neelum | 12 | 29.70 |
| 3      | 30.103769 | 78.002029 | Kudladampatti | ILtDM3 | Neelum | 15 | 27.86 |
| 4      | 30.103239 | 78.106822 | Palamedu | ILtDM4 | Neelum | 15 | 43.75 |
| 5      | 30.124304 | 77.592151 | HC&RI (PKM) | ILtDM5 | Neelum | 16 | 18.47 |
| 6      | 30.049360 | 77.86057 | Melangalam | ILtDM6 | Neelum | 29 | 9.61 |
| 7      | 30.179918 | 77.538597 | Kumbakarai | ILtDM7 | Neelum | 18 | 41.80 |
| 8      | 30.138443 | 77.519532 | Vadagara | ILtDM8 | Vadumangai | 21 | 13.15 |
| 9      | 30.256757 | 78.126674 | Gopalpatti | ILtDM9 | Neelum | 4 | 41.93 |
| 10     | 30.220502 | 78.161245 | Mulaiyur | ILtDM10 | Neelum | 13 | 55.84 |
| 11     | 30.232345 | 78.219566 | Velanpatti | ILtDM11 | Neelum | 14 | 28.35 |
| 12     | 30.186534 | 77.799800 | Nuthalapuram | ILtDM12 | Neelum | 6 | 14.10 |
| 13     | 30.167489 | 78.557565 | Chandrapuram | ILtDM13 | Neelum | 16 | 26.47 |
| 14     | 30.070105 | 78.476869 | Mobripatti | ILtDM14 | Neelum | 10 | 46.51 |
| 15     | 30.130029 | 78.414420 | Mottaiyappathi | ILtDM15 | Neelum | 14 | 46.03 |
| 16     | 30.208179 | 78.060184 | Madhehalli | ILtDM16 | Neelum | 9 | 32.69 |
| 17     | 30.318716 | 78.477068 | Ettipatti | ILtDM17 | Neelum | 24 | 8.95 |
| 18     | 30.354418 | 78.532969 | Vadunganur | ILtDM18 | Neelum | 16 | 16.75 |
| 19     | 30.351093 | 78.582652 | Andiyur | ILtDM19 | Neelum | 17 | 53.84 |
| 20     | 30.201931 | 78.601207 | Nadupatti | ILtDM20 | Neelum | 23 | 21.80 |
| 21     | 30.757643 | 78.041693 | Palbakkai | ILtDM21 | Neelum | 24 | 30.50 |
| 22     | 30.797774 | 78.025106 | Semmadiapatti | ILtDM22 | Neelum | 27 | 14.50 |
| 23     | 30.785663 | 77.871943 | Mangamethai | ILtDM23 | Neelum | 21 | 25.60 |
| 24     | 30.784840 | 77.872081 | Veerakkal | ILtDM24 | Neelum | 19 | 26.50 |
| 25     | 30.781436 | 79.431387 | Sennalery | Vellore | ILtDM25 | Neelum | 25 | 22.20 |
| 26     | 30.388515 | 78.591824 | Chinnaarampatti | ILtDM26 | Neelum | 20 | 33.52 |
| 27     | 30.408924 | 78.590542 | Odayamuthur | ILtDM27 | Neelum | 18 | 36.15 |
| 28     | 30.359949 | 78.387436 | Lekkanayakanpatti | ILtDM28 | Neelum | 25 | 29.00 |
| 29     | 30.290105 | 78.394796 | Nagamangalam | ILtDM29 | Neelum | 23 | 34.00 |
| 30     | 30.162863 | 77.405140 | Puliyangudi | ILtDM30 | Neelum | 12 | 31.03 |
### Table 2: Mycelial characters of isolates of *L. theobromae*

| S. No. | Isolate Code | Colour                | Reverse          | Topography | Margin      | Zonation     | Days to cover Petri Dish (9 cm) * |
|--------|--------------|-----------------------|------------------|------------|-------------|--------------|----------------------------------|
| 1      | ILtDM1       | Greyish white         | Black            | Aerial     | Irregular   | No           | 3                                |
| 2      | ILtDM2       | Grey                  | Black            | Aerial     | Irregular   | No           | 3                                |
| 3      | ILtDM3       | White                 | Dark green to black | Fluffy   | Smooth      | No           | 5                                |
| 4      | ILtDM4       | White                 | Dark green to black | Aerial   | Irregular   | No           | 2                                |
| 5      | ILtDM5       | Blackish grey         | Dark black       | Aerial     | Irregular   | No           | 3                                |
| 6      | ILtDM6       | Greyish black         | Dark black       | Aerial     | Irregular   | No           | 3                                |
| 7      | ILtDM7       | Greyish white         | Black            | Aerial     | Irregular   | No           | 3                                |
| 8      | ILtDM8       | Grey                  | Black            | Aerial     | Irregular   | No           | 3                                |
| 9      | ILtDM9       | Greyish black         | Dark black       | Aerial     | Irregular   | No           | 2                                |
| 10     | ILtDM10      | Grey                  | Black            | Aerial     | Irregular   | No           | 3                                |
| 11     | ILtDM11      | White                 | Dark green to black | Aerial   | Irregular   | No           | 2                                |
| 12     | ILtDM12      | Greyish white         | Black            | Aerial     | Irregular   | No           | 2                                |
| 13     | ILtDM13      | Greyish white         | Black            | Aerial     | Irregular   | No           | 3                                |
| 14     | ILtDM14      | White                 | Dark green to black | Aerial   | Irregular   | No           | 4                                |
| 15     | ILtDM15      | Dark grey             | Black            | Aerial     | Irregular   | No           | 3                                |
| 16     | ILtDM16      | Grey                  | Black            | Aerial     | Irregular   | No           | 2                                |
| 17     | ILtDM17      | Blackish grey         | Dark black       | Flat       | Smooth      | No           | 3                                |
| 18     | ILtDM18      | Dark grey             | Black            | Aerial     | Irregular   | No           | 3                                |
| 19     | ILtDM19      | Greyish white         | Black            | Aerial     | Irregular   | No           | 3                                |
| 20     | ILtDM20      | Dark grey             | Black            | Fluffy     | Smooth      | No           | 3                                |
| 21     | ILtDM21      | White                 | Dark green to black | Aerial   | Irregular   | No           | 3                                |
| 22     | ILtDM22      | Dark grey             | Black            | Aerial     | Irregular   | No           | 3                                |
| 23     | ILtDM23      | Greyish black         | Dark black       | Aerial     | Irregular   | No           | 2                                |
| 24     | ILtDM24      | Greyish white         | Black            | Aerial     | Irregular   | No           | 3                                |
| 25     | ILtDM25      | White                 | Dark green to black | Aerial   | Irregular   | No           | 3                                |
| 26     | ILtDM26      | Greyish black         | Dark black       | Aerial     | Irregular   | No           | 2                                |
| 27     | ILtDM27      | Greyish white         | Black            | Aerial     | Irregular   | No           | 3                                |
| 28     | ILtDM28      | Greyish white         | Black            | Aerial     | Irregular   | No           | 2                                |
| 29     | ILtDM29      | Greyish black         | Black            | Aerial     | Irregular   | Concentric zonation | 3                                |
| 30     | ILtDM30      | Blackish grey         | Dark black       | Aerial     | Irregular   | No           | 2                                |

*Mean values of three replications*
**Table 3** Spore characters of isolates of *L. theobromae*

| S. No. | Isolate Code | Pycnidia production* | Shape of the conidia | Conidia production* | Size of conidia (µm)** |
|--------|--------------|----------------------|----------------------|----------------------|------------------------|
|        |              |                      |                      |                      | Length | Breadth   |
| 1      | ILtDM 1      | ++                   | Ellipsoid            | ++                   | 22.0   | 13.6      |
| 2      | ILtDM 2      | ++                   | Ellipsoid            | +                    | 23.8   | 13.6      |
| 3      | ILtDM 3      | ++                   | Ellipsoid            | +                    | 23.2   | 13.6      |
| 4      | ILtDM 4      | +                    | Ellipsoid            | +                    | 20.0   | 11.0      |
| 5      | ILtDM 5      | ++                   | Ellipsoid            | +++                  | 26.8   | 13.0      |
| 6      | ILtDM 6      | +++                  | Ellipsoid            | +++                  | 18.4   | 10.6      |
| 7      | ILtDM 7      | ++                   | Ellipsoid            | +                    | 23.2   | 13.2      |
| 8      | ILtDM 8      | +                    | Ellipsoid            | +                    | 22.6   | 11.4      |
| 9      | ILtDM 9      | +++                  | Ellipsoid            | +++                  | 23.8   | 12.0      |
| 10     | ILtDM 10     | ++                   | Ellipsoid            | +++                  | 24.0   | 12.6      |
| 11     | ILtDM 11     | +                    | Ellipsoid            | +                    | 24.0   | 12.0      |
| 12     | ILtDM 12     | ++                   | Ellipsoid            | ++                   | 22.6   | 12.0      |
| 13     | ILtDM 13     | +++                  | Ellipsoid            | +++                  | 23.8   | 13.6      |
| 14     | ILtDM 14     | +                    | Ellipsoid            | +                    | 20.2   | 12.8      |
| 15     | ILtDM 15     | +                    | Ellipsoid            | +                    | 21.6   | 13.4      |
| 16     | ILtDM 16     | +++                  | Ellipsoid            | +++                  | 24.0   | 14.2      |
| 17     | ILtDM 17     | +                    | Ellipsoid            | +                    | 25.0   | 11.8      |
| 18     | ILtDM 18     | +                    | Ellipsoid            | +                    | 24.4   | 13.4      |
| 19     | ILtDM 19     | ++                   | Ellipsoid            | ++                   | 23.6   | 14.0      |
| 20     | ILtDM 20     | +                    | Ellipsoid            | +                    | 20.4   | 11.0      |
| 21     | ILtDM 21     | ++                   | Ellipsoid            | +                    | 22.6   | 12.8      |
| 22     | ILtDM 22     | +                    | Ellipsoid            | +                    | 21.4   | 13.0      |
| 23     | ILtDM 23     | +                    | Ellipsoid            | +                    | 24.8   | 12.8      |
| 24     | ILtDM 24     | +                    | Ellipsoid            | +                    | 23.6   | 13.6      |
| 25     | ILtDM 25     | +                    | Ellipsoid            | +                    | 20.2   | 11.6      |
| 26     | ILtDM 26     | +                    | Ellipsoid            | +                    | 23.8   | 13.8      |
| 27     | ILtDM 27     | ++                   | Ellipsoid            | +                    | 21.6   | 11.2      |
| 28     | ILtDM 28     | ++                   | Ellipsoid            | +                    | 23.2   | 13.6      |
| 29     | ILtDM 29     | ++                   | Ellipsoid            | +                    | 20.2   | 12.6      |
| 30     | ILtDM 30     | +++                  | Ellipsoid            | +++                  | 22.6   | 13.2      |

* Mean values of three replications; ** Mean values of five replications
- no, + poor, ++ medium, +++ good

**Fig. 1** Mango dieback disease incidence caused by different isolates of *Lasiodiplodia theobromae*
Plate.1 Symptomatology of Mango dieback disease

Plate.2 Variability among mycelial and conidial character

Variability in morphological characters

Variability in mycelial characters

Mycelial growth of the pathogen, *L. theobromae* was observed to be hyaline to white coloured initially, which radiated from the small piece of the infected tissue. Upon the maturation of mycelium, it exhibited the colour variation ranging from grey to dark greyish black colour. Pycnidia were observed to be scattered along the periphery of the Petri dish (Table 2).

The colour of matured culture was grey to dark greyish black. Among the isolates greyish white mycelium was produced by the isolates *viz.*, ILtDM1, ILtDM7, ILtDM12, ILtDM19, ILtDM24, ILtDM27, ILtDM28. The isolates *viz.*, ILtDM2, ILtDM8, ILtDM10, ILtDM16 produced grey colour mycelium and ILtDM3, ILtDM4, ILtDM11, ILtDM14, ILtDM21, ILtDM25 produced white coloured mycelium. Blackish grey mycelium was produced by the isolates *viz.*, ILtDM5, ILtDM17, ILtDM30. The isolates ILtDM6, ILtDM9, ILtDM23, ILtDM26, ILtDM29 produced greyish black mycelium and ILtDM15, ILtDM18, ILtDM20, ILtDM22 produced dark grey coloured mycelium (Plate. 2).

Apart from these, isolates of ILtDM5, ILtDM6, ILtDM9, ILtDM17, ILtDM23,
ILtDM26, ILtDM30 produced dark black pigmentation, ILtDM3, ILtDM4, ILtDM11, ILtDM14, ILtDM21 and ILtDM25 produced dark green to black pigmentation and all others produced black coloured pigmentation.

The pathogenic isolates exhibited three different topographic features viz., aerial, flat and fluffy growth. The isolate ILtDM17 only had flat growth, ILtDM3, ILtDM20 had fluffy growth and remaining all had aerial growth. Margin of different isolates were characterized as smooth and irregular. All isolates produced irregular margin except ILtDM3, ILtDM17, ILtDM20 which produced smooth margin. But the isolate, ILtDM29 alone produced concentric zonation, which was not observed in other isolates.

The isolates ILtDM4, ILtDM9, ILtDM11, ILtDM12, ILtDM16, ILtDM23, ILtDM26, ILtDM28 and ILtDM30 were grown fast and took two days to cover the 9 cm diameter Petri dish followed by ILtDM1, ILtDM2, ILtDM5, ILtDM6, ILtDM7, ILtDM8, ILtDM10, ILtDM13, ILtDM15, ILtDM17, ILtDM18, ILtDM19, ILtDM20, ILtDM21, ILtDM22, ILtDM24, ILtDM25, ILtDM27, and ILtDM29. These isolates required three days to cover the Petri dish and ILtDM14 required four days. Among all these, the isolate ILtDM3 was very slow and took five days to cover the Petri dish. In this study, culture characters of all the isolates were agreed with the findings of the authors (Goos et al., 1961; Punithalingam, 1976; Ko et al., 2004; Shah et al., 2010; Sathya et al., 2017; Ekanayake et al., 2019).

**Variability in spore characters**

Morphological variations in pycnidium and spores of *L. theobromae* were studied (Table 3). Pycnidia were black and scattered along the periphery of the Petri Dish. Among 30 the isolates, isolates ILtDM6, ILtDM9, ILtDM13, ILtDM16 and ILtDM30 had more production of pycnidia and sporulation than other isolates. Conidia were initially hyaline, unicellular, sub ovoid to ellipsoid in shape and mature conidia were dark brown to black colour, bi-celled, thick walled, ellipsoidal shape with longitudinal striations (Plate.2). The size of the spore (length and breadth) was in the range between 18.4 - 26.8 x 10.6 - 14.2 µm. Among the isolates, ILtDM5 had the highest length of the spore and the smallest spore length was recorded in with ILtDM6. Such variation in conidial characters was reported by different workers, which was attributed to the inherent genetic variability (Khanzada et al., 2004; Alves et al., 2008; Shah et al., 2010; Phillips et al., 2013).

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