

Original Research Article

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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 discolouration, 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
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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).

$$I (\%) = (ni/N) \times 100$$

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 4⁰C 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.	Location		Village	District	Isolate Code	Variety	Age of trees (Years)	Disease Incidence (%)
	Latitude	Longitude						
1	9.975802	78.206805	AC&RI (MDU)	Madurai	ILtDM1	Bangalora	10	12.90
2	9.994748	77.974122	Melakkal		ILtDM2	Neelum	12	29.70
3	10.103769	78.002029	Kutladampatti		ILtDM3	Alphonso	15	27.86
4	10.103239	78.106822	Palamedu		ILtDM4	Bangalora	15	43.75
5	10.124304	77.592151	HC&RI (PKM)	Theni	ILtDM5	Bangalora	16	18.47
6	10.049305	77.586057	Melmangalam		ILtDM6	Bangalora	29	9.61
7	10.179918	77.538597	Kumbakarai		ILtDM7	Neelum	18	41.80
8	10.138443	77.519532	Vadagarai		ILtDM8	Vadumangai	21	13.15
9	10.256757	78.126674	Gopalpatti	Dindigul	ILtDM9	Bangalora	4	41.93
10	10.220502	78.161245	Mulaiyur		ILtDM10	Bangalora	13	55.84
11	10.232345	78.219566	Velanpatti		ILtDM11	Banganapalli	14	28.35
12	10.186534	77.799800	Nuthulapuram		ILtDM12	Alphonso	6	14.10
13	12.167489	78.557565	Chandrapuram	Dharmapuri	ILtDM13	Bangalora	16	26.47
14	12.070105	78.476869	Mobripatti		ILtDM14	Bangalora	10	46.51
15	12.130029	78.414420	Mottaiyanpatti		ILtDM15	Neelum	14	46.03
16	12.208179	78.060184	Madhehalli		ILtDM16	Malgoa	9	32.69
17	12.318716	78.477068	Ettipatti	Krishnagiri	ILtDM17	Alphonso	24	8.95
18	12.354418	78.532969	Vaduganur		ILtDM18	Banganapalli	16	16.75
19	12.351093	78.582652	Andiyur		ILtDM19	Bangalora	17	53.84
20	12.201931	78.601207	Nadupatti		ILtDM20	Bangalora	23	21.80
21	11.757643	78.041693	Palbakki	Salem	ILtDM21	Salemgundu	24	30.50
22	11.797774	78.025106	Semmadapatti		ILtDM22	Bangalora	27	14.50
23	11.785663	77.871943	Mangamethai		ILtDM23	Neelum	21	25.60
24	11.784840	77.872081	Veerakkal		ILtDM24	Bangalora	19	26.50
25	12.781436	79.431387	Sennaleri	Vellore	ILtDM25	Bangalora	25	22.20
26	12.388515	78.591824	Chinnarampatti	Tiruppattur	ILtDM26	Alphonso	20	33.52
27	12.408924	78.590542	Odayamuthur		ILtDM27	Neelum	18	36.15
28	10.359949	78.387436	Lekkanayakkanpatti	Trichy	ILtDM28	Neelum	25	29.00
29	10.290105	78.394796	Nagamangalam		ILtDM29	Bangalora	23	34.00
30	9.162863	77.405140	Puliyangudi	Tenkasi	ILtDM30	Neelum	12	31.03

Table.2 Mycelial characters of isolates of *L. theobromae*

S. No.	Isolate Code	Colour		Topography	Margin	Zonation	Days to cover Petri Dish (9 cm) *
		Observe	Reverse				
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*

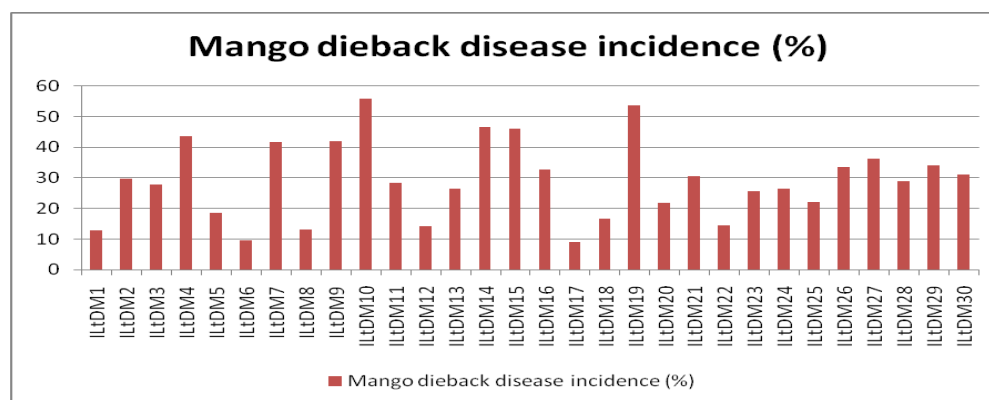


Plate.1 Symptomatology of Mango dieback disease

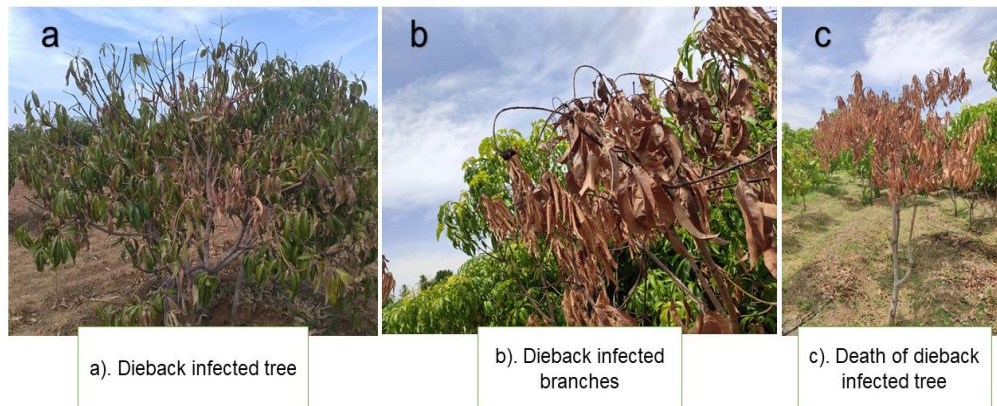


Plate.2 Variability among mycelial and conidial character



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 × 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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