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SURVEY AND CHARACTERIZATION OF FUNGAL PATHOGENS ASSOCIATED WITH LEAF SPOT AND BLIGHT OF COCONUT SEEDLINGS IN NURSERIES

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ABSTRACT

Leaf spot and blight disease in coconut nursery is an important disease that adversely affect the survival of seedlings. The symptomatology indicating with irregular brown necrotic spots at initial stage and at sever stages these spots get coalesce which leads to drying and blighting of leaf tip or margins. Surveys were conducted for the assessment of leaf spot and blight in five major coconut growing areas of Andhra Pradesh. Eleven isolates collected from these major growing coconut areas, percent incidence of leaf spot and seedling blight ranging from 7.50% to 18.25 % in surveyed areas and maximum incidence was recorded in Pandirimamidi area (18.25%). Among these eleven collected isolates the West Godavari ,Palakollu isolate has shown the maximum lesion diameter of 28.9mm and it was proved to be virulent. The texture of the colony varied from fluffy, suppressed to raised. The colour of the colony at initial stage was white and at later stages it varies from greyish white to greyish black. The growth rate varies from 22.38 mm/day to 29.00mm/day. All the eleven isolates have showed variation in the morphological characters. These isolates showing the amplicon size of 550 bp and the fungal pathogen of leaf spot and blight disease of coconut nursery seedlings was identified as *Lasiodiplodia theobromae* based on its morphological and molecular characters.

Key words : Leaf spot and blight, Pathogen isolation, Pathogenicity testing, Morphological characterization, Molecular identification.

Introduction

Coconut (*Cocos nucifera* L.) is one of the most important perennial plantation crops cultivated across tropical and subtropical regions of the world. Belonging to the family *Arecaceae*, it is often referred to as the “tree of life” because almost every part of the palm has economic, nutritional, or industrial value.

The producers of coconut include from agribusinesses to smallholders by bringing the socio-economic stability in lives of millions of farmers, particularly in countries such as India, Indonesia, the Philippines, and Sri Lanka. It contributes significantly to food, fuel, fibre, and shelter, in addition to supporting large industries like copra processing, coconut oil extraction, coir manufacturing, beverages, and value-added products. The coconut palm cultivation generates raw materials for different uses from handicrafts to industrial uses (Luisito Penamora, 2007).

As it is being perennial, it is affected by many pests and diseases. More than 70 fungi are associated with leaf diseases in coconut (Keerthana *et al.*, 2023). The leaf spot and blights are noticed in almost all major coconut growing areas in Andhra Pradesh. The infection often occurs in lower leaves of seedlings from apical leaflets as it is being systemic it invades rachis to petiole, later creating the dryness of leaflets.

As these diseases are not fatal, they also make a considerable damage to the neglected plantations at advanced stages of infection. By decreasing the area of photosynthesis which leads to decrease in yield and the general vitality of coconut.

Materials and Methods

The nurseries in five districts of Andhra Pradesh *viz.* Alluri seetharamaraju, Eluru, East Godavari, West Godavari and Dr. B.R. Ambedkar konaseema districts were surveyed during 2025-26 and leaf spot and blight disease of coconut seedlings were studied and information were recorded (Table 1). The most of the nurseries that surveyed contain the varieties and hybrids of East Coast Tall (ECT) and Godavari Ganga (GG) respectively and in Coconut development board there are few more varieties that surveyed are Ganaga Bondam (GB), Pratap, Malayan Yellow Dwarf (MYD). During the survey in coconut nurseries, total number of coconut seedlings found in nurseries were counted. Then 100 seedlings were selected randomly from each of the nurseries. This sampling intensity has been successfully used in coconut and oil palm nursery disease surveys (Khan and Hossain, 2013; Lekete *et al.*, 2019). Each seedling was observed carefully and disease symptoms were recorded. Disease incidence was assessed (Table 2) as percentage of plants infected with at least one leaf spot or visible symptoms.

Disease identification is done by following Ramjegathesh *et al.* (2019)

Leaf Spot Symptoms

- Small, circular to irregular brown or black necrotic lesions.
- Presence of chlorotic halos.
- Lesions may coalesce under severe infection.

Seedling Blight Symptoms

- Rapid expansion and coalescence of lesions
- Drying and blighting of leaf tips or margins.
- Severe cases result in partial and complete leaf necrosis.

Disease Incidence (%) = (Number of diseased plants / Total number of plants observed) × 100.

Disease severity assessment: Disease severity scale will be assessed using the scale as described (Khan and Hossain, 2013).

Scale	Description
0	No visible symptoms
1	1 - 5 % leaf area diseased
2	6 - 12% leaf area diseased
3	13– 25 % leaf area diseased
4	26- 50 % leaf area diseased
5	>50 % leaf area diseased

During the months of December to March random surveys were carried out. Percentage of disease incidence was calculated from infected plants by total number of plants from nursery where disease suspected plants were counted (Total 100 plants taken, healthy and diseased plants). Approximately four to five diseased leaf samples were collected in polythene bags, labelled with information and carried for further studies to the laboratory.

Based on numerical data obtained in respect of total number of plants and diseased plants per field were surveyed, the incidence of diseased per nursery was calculated using the following formula.

The incidence of disease was estimated using the following equation.

Disease incidence (%) : Total number of diseased plants/Total number of plants observed × 100

Disease severity assessment is done according to the scale described (Khan and Hossain, 2013) later these disease severity scores were converted into the Percent Disease intensity.

$$PDI = \frac{\Sigma(\text{Disease score} \times \text{Number of seedlings in that score})}{\text{Maximum disease score} \times \text{Total number of seedlings observed}} \times 100$$

Collection of samples

Following the survey conducted in the major coconut growing regions, samples showing typical symptoms of leaf spot and blight were collected for further laboratory investigations. Symptomatic leaf samples exhibiting characteristic lesions were carefully collected from different coconut nurseries representing the surveyed locations. The samples were placed individually in clean, labelled polythene bags and transported to the laboratory for isolation and identification of the associated pathogen. The collected samples were subsequently processed for isolation, cultural and morphological characterization, pathogenicity testing and molecular identification of the associated pathogen.

Table 1 : Assessment of percent incidence, PDI and Disease scores of every surveyed area.

S. no.	Name of Nursery/ Research Station	Village	Mandal	District	Total no. of seedlings	Age of seedlings	Variety/ Hybrid	Percent incidence (%)	PDI (%)	Disease score
1	Horticultural Research Station	Pandirimamidi	Rampachodavaram	Alluri Seetharamaraju	1400	8 months	ECT	18.25%	52.90	5
2	Aditya Nursery	Kadiyam	Kadiyam	East Godavari	2000	6 months	ECT	7.50%	40.53	4
3	Sri Lakshmi Ganapathi Nursery	Kadiyam	Kadiyam	East Godavari	3000	6 months	ECT	15.75%	40.20	4
4	Coconut development board	Vegiwada	Pedavegi	Eluru	50000	6 months	ECT,GG, MYD,GB,Pratap	17.50%	37.20	4
5	Horticultural Research Station	Vijayrai	Pedavegi	Eluru	2500	8 months	ECT	14.75%	54.80	5
6	Horticultural Research Station	Ambaipeta	Ambajipeta	Dr. B.R Ambedkar Konaseema	40000	6 months	ECT,GG	14.25%	25.8	3
7	DS Gardens	Mandapalli	Kotapeta	Dr. B.R Ambedkar Konaseema	10000	6 months	ECT,GG	7.50%	25.99	4
8	Konaseema Udyana Nursery	Ambajipeta	Ambajipeta	Dr. B.R Ambedkar Konaseema	4000	6 months	ECT,GG	14.30%	34.90	4
9	Krishna Chandra Nursery	Thondavaram	Ambajipeta	Dr. B.R Ambedkar Konaseema	2000	8 months	ECT	9.25%	35.23	4
10	Mallampalli Nageshwara rao nursery	Palakollu	Palakollu	West Godavari	3500	6 months	ECT	4.50%	20.77	3
11	Radha Krishna Nursery	Palakollu	Palakollu	West Godavari	3000	8 months	ECT	6.50%	23.75	3

Isolation of pathogen from suspected leaf spot and blight symptoms

Isolation from fungal leaf spots is done commonly by making into small pieces from the margin with lesions and are surface-sterilized, washed in sterile water, blotted dry and aseptically placed on a PDA. The plates are incubated at about 25°C for 3–5 days to allow fungal growth.

The fungal isolate grown is sub-cultured onto a new medium and incubated for five days. An inoculation with 100-500 fungal spores are transferred onto PDA medium by sterilized scalpel. The inoculation will be spread by glass spreaders on the surface of the medium using 0.1 ml distilled water. The plates are left inside the laminar air flow for 10 min to dry. The inoculated media is to be incubated at 28^0C for 16 – 18 hours. Therefore, one germinated spore is selected and transformed into new PDA medium. The fungal growth will be recorded after seven days of incubation (Noman *et al*., 2017).

Pathogenicity test

A representative highly virulent isolate was selected based on cultural characterization. The collected fungal isolates were evaluated for pathogenicity on seedlings of the East Coast Tall (ECT) variety of coconut, as the majority of the survey was conducted in this variety. The pathogenicity test was carried out under screen-house conditions (Ayika *et al*., 2026; Prathibha *et al*., 2024).

Initially, the leaf surface was sterilized using sodium hypochlorite. The leaf tips were then pin-pricked to facilitate

pathogen entry. A 5-mm-diameter mycelial disc was cut from the actively growing region of the fungal culture plate and placed over the pin-pricked area. The inoculated leaf tips were covered with moist cotton swabs to maintain adequate humidity and subsequently enclosed in polythene bags to provide favourable conditions for infection (Ramjegathesh *et al*., 2019).

Three replications were maintained for each test isolate. Symptoms began to appear on the seventh day after inoculation, following an observation period of six days. After removing the cotton swabs, brownish-black necrotic lesions were observed on the inoculated leaf tips. The virulence of the isolates was assessed based on the time taken for symptom appearance following inoculation. Isolates that produced symptoms earlier were considered more virulent than those that exhibited symptoms later.

Cultural and Morphological characterization

The total of all eleven isolates were grown on the PDA medium to study their growth and variability in colony morphological and cultural characters. The fungal disc of 5mm was cut by the cork borer from the eight day old culture plates and this was placed at the center of the each sterile petri dish (90 mm) containing PDA medium. These plates were incubated at the room temperature (25±2) for about seven days. The cultural characters like colony colour, growth rate (mm per day), texture, growth pattern, sporulation and the morphological characters like the fruiting body, shape, septation and colour of spores were also recorded.

Molecular characterization

The virulence of the fungal isolates was assessed based on mycelial radial growth, pycnidia production, and pycnidiospore production. Among the isolates evaluated, LSSB-1, LSSB-2, LSSB-10 and LSSB-11 produced the highest numbers of pycnidia and pycnidiospores within a relatively short period. These isolates also exhibited a greater capacity to infect healthy coconut seedlings, indicating their higher virulence. Based on these findings, the highly virulent isolates were selected for molecular identification of the leaf spot and blight-causing pathogen. Genomic DNA was extracted using the cetyltrimethylammonium bromide (CTAB) method, following the protocol described by Ma *et al*. (2001).

DNA extraction

Approximately 1 g of mycelium from each 7-day-old fungal isolate was transferred into a sterile 1.5 mL Eppendorf tube. The mycelium was homogenized in 400 µL of sterile extraction buffer containing 200 mM Tris-HCl, 250 mM NaCl, 25 mM EDTA and 0.5% SDS. Genomic DNA was extracted following the protocol described by Noman *et al*. (2021). The resulting DNA pellet was resuspended in 100 µL of 1× TE buffer (10 mM Tris-HCl and 1 mM EDTA, pH 8.0) and incubated overnight at 4°C to facilitate complete dissolution. The extracted DNA samples were stored at –20°C until further analysis. DNA concentration was measured at 260 nm using a Nano Drop spectrophotometer, while DNA quality was assessed by electrophoresis on a 1% agarose gel prior to PCR amplification.

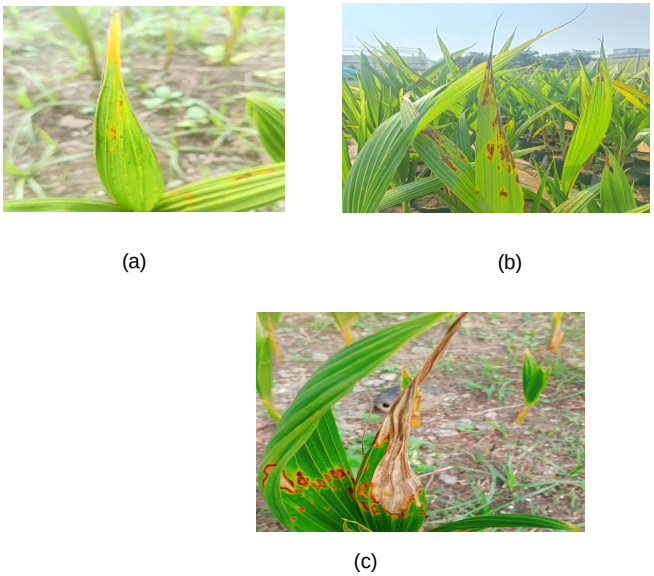


Fig. 1 : (a) Small circular to irregular brown or black necrotic lesions, (b) Rapid expansion and coalescence of lesions, (c) Drying and blighting of leaf tips or margins at severe cases.

PCR amplification and sequencing

The internal transcribed spacer (ITS) regions of the isolates were amplified using the forward primer ITS1 (5'-CTTGGTCATTTAGAGGAAGTA-3') and the reverse primer ITS4 (5'-TCCTCCGCTTATTGATAGC-3') (White *et al*., 1990). Each 25 µl PCR reaction mixture contained 2.0 µl of template DNA, 2.5 µl PCR buffer, each dNTPs 0.5 µl, $MgCl_2$ 1.5 µl ,Taq 1 µl, DNA 2 µl, Forward and reverse each of 1 µl respectively and 14 µl of nuclease-free water. The reaction mixture was thoroughly mixed before thermal cycling, which included an initial denaturation at 94°C for 5 minutes, followed by 30 cycles of denaturation at 94°C for 30 seconds, annealing at 55°C for 30 seconds, and extension at 72°C for 30 seconds (Ashokkumar *et al*., 2018). A final extension step at 72°C for 5 minutes was included, ending with a hold at 4°C. Successful amplification of the ITS regions was verified by electrophoresis on a 1% agarose gel with compared to a 100 bp DNA ladder (GeNei

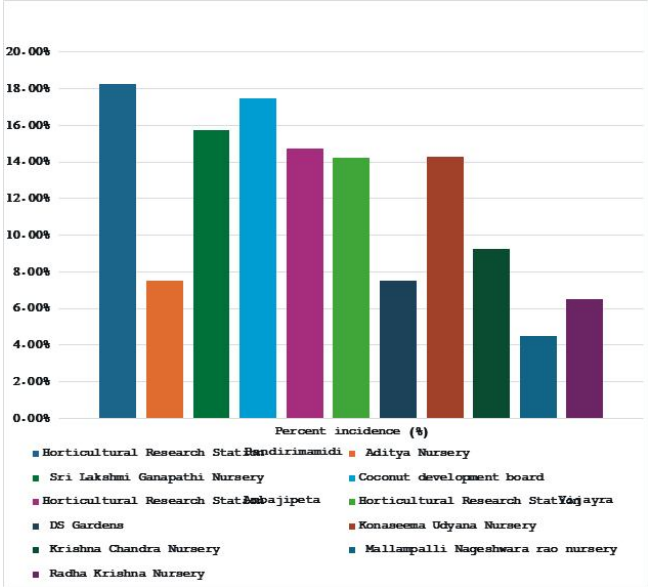


Fig. 2 : Graphical representation of the percent incidence in the major coconut growing districts of Andhra Pradesh.



Fig. 3 : Pathogenicity test of isolated pathogen associated with leaf spot and blight of coconut under screen house conditions. Total of 11 isolates each with three replications were maintained along with three control seedlings (C1, C2, C3).

Laboratories Pvt Ltd), Purified PCR products were sequenced.

Sequence analysis and phylogenetic tree construction

The nucleotide homogeneity of the obtained consensus sequences was evaluated by comparing them with other sequences in the GenBank database using the BLASTn tool (http://www.ncbi.nlm.nih.gov/BLAST) and these sequences were subsequently deposited in the GenBank database. A phylogenetic tree was constructed using the neighbour-joining method within the same software, and branch support was evaluated using 1000 bootstrap replicate.

Results and Discussion

Survey

Survey was conducted during 2025 December to 2026 March in the five major coconut growing areas to record the prevalence of leaf spot and seedling blight.



Fig. 4 : Straw coloured lesions were developed after 6 days of incubation period.

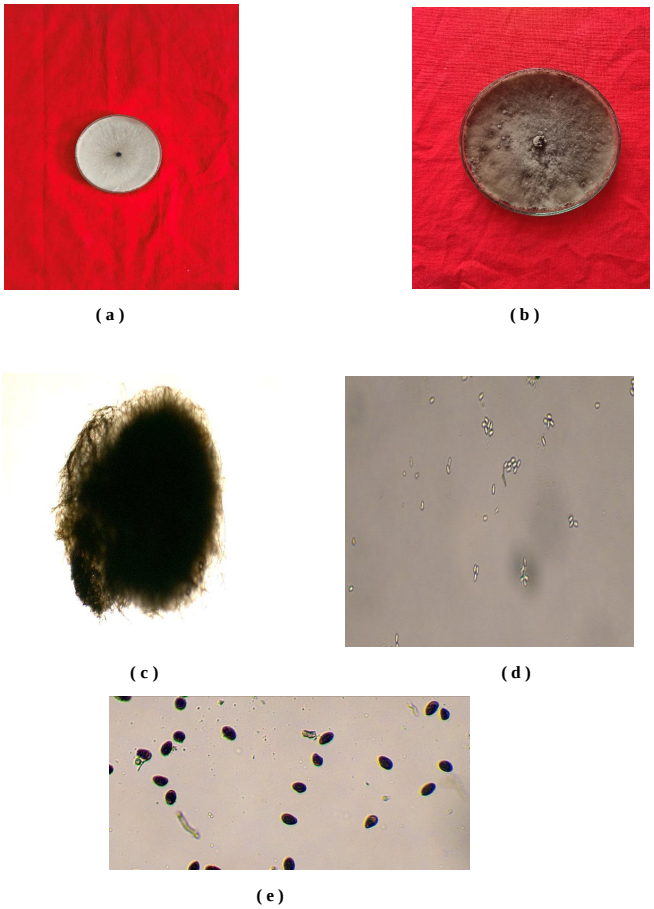


Fig. 5 : **(a)** Initially colony colour, **(b)** Mature colony colour, **(c)** Pycnidia- Fruiting body, **(d)** Hyaline young aseptate pycnidiospores, **(e)** Dark brown matured septate pycnidiospore.

Alluri Seetharamaraju (Pandirimamidi), East Godavari (Kadiyam), West Godavari (Palakollu), Eluru (Vijayrai, Vegivada) and Dr.B.R Ambedkar Konaseema (Ambajipeta, Tondavaram, Kothapeta) and disease index was calculated by Johnson *et al.* (2014).

Leafspot and seedling blight was recorded in all the areas that surveyed and the incidence ranged from 20.77 to 52.90 percent. The maximum incidence was recorded

Table 2 : Prevalence of the disease in the surveyed districts of Andhra Pradesh.

S. no.	District	Name of Nursery/ Research Station	Village	Mandal	Percent incidence (%)	PDI (%)
1	Alluri Seetharamaraju	Horticultural Research Station	Pandirimamidi	Rampachodavarm	18.25%	52.90
	Mandal mean				18.25%	52.90
	District mean				18.25%	52.90
2	East Godavari	Aditya Nursery	Kadiyam	Kadiyam	7.50%	40.53
3	East Godavari	Sri Lakshmi Ganapathi Nursery	Kadiyam	Kadiyam	15.75%	40.20
	Mandal mean				11.62%	40.36
	District mean				11.62%	40.36
4	Eluru	Coconut development board	Vegiwada	Pedavegi	17.50%	37.20
5	Eluru	Horticultural Research Station	Vijayrai	Pedavegi	14.75%	54.80
	Mandal mean				16.12%	46.00
	District mean				16.12%	46.00
6	Dr.B.R Ambedkar Konaseema	DS Gardens	Mandapalli	Kotapeta	7.50%	25.99
	Mandal mean				7.50%	25.99
7	Dr. B.R Ambedkar Konaseema	Horticultural Research Station	Ambaipeta	Ambaipeta	14.25%	25.80
8	Dr. B.R Ambedkar Konaseema	Konaseema Udyana Nursery	Ambajipeta	Ambaipeta	14.30%	34.90
9	Dr. B.R Ambedkar Konaseema	Krishna Chandra Nursery	Thondavaram	Ambaipeta	9.25%	35.23
	Mandal mean				12.6%	31.97
	District mean					
10	West Godavari	Mallampalli Nageshwara Rao Nursery	Palakollu	Palakollu	4.50%	20.77
11	West Godavari	Radha Krishna Nursery	Palakollu	Palakollu	6.50%	23.75
	Mandal mean				5.5%	22.26
	District mean				5.5%	22.26

at Alluri Seethramaraju district (52.90 per cent) represented in Table 2.

Pathogenicity test

All the eleven isolates were artificially inoculated on the coconut seedlings and observed for their virulence activity. The results of pathogenicity test indicated that, among all the isolates the isolate LSSB-11 collected from the West Godavari, Palakollu produced the maximum lesion length diameter of 28.9 mm followed by LSSB-10 and LSSB-1 collected from West Godavari and Alluri Seethramaraju districts. The isolate collected from Kothapet (LSSB-7) produced minimum lesion diameter of 13.3mm (Table 3). The coconut seedlings which are inoculated with mycelial disc exhibit the straw coloured lesions on the inoculated areas after 6 days of incubation period (Surulirajan *et al*., 2014) in Fig. 3.

Cultural characterization

The isolates were grown in PDA medium to study

Table 3 : Pathogenicity test.

Name of isolate	Mean lesion diameter(mm)	Incubation period	Virulency
LSSB-1	27.80	6 Days	Virulent
LSSB-2	26.10	6 Days	Virulent
LSSB-3	17.80	7 days	Modarately virulent
LSSB-4	17.80	7 days	Modarately virulent
LSSB-5	15.00	8 days	Less virulent
LSSB-6	15.00	7 days	Modarately virulent
LSSB-7	13.30	8 days	Less virulent
LSSB-8	13.90	8 days	Less virulent
LSSB-9	18.30	7 days	Modarately virulent
LSSB-10	27.80	6 Days	virulent
LSSB-11	28.90	6 Days	Highly virulent

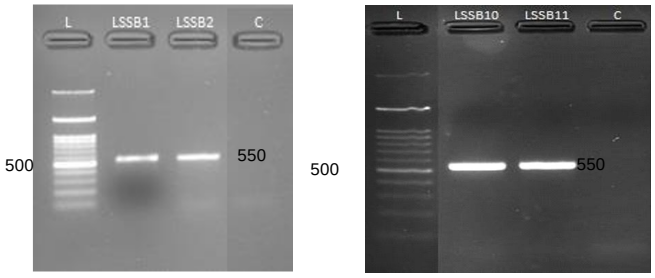


Fig. 6 : PCR amplification of r RNA- ITS region of the isolates of *L. theobromae*. L – Representing the 100 bp ladder, C- Control.

Table 4 : Cultural characteristics.

Name of isolate	Initial colony colour	Mature colony colour	Texture	Growth pattern	Sporulation ($\times 10^5$)	Growth (mm/day)
LSSB-1	White	Greyish black	Dense, cottony	Very Rapid,radial	3.26	28.33
LSSB-2	White	Greyish black	Raised, uniform, cottony	Very Rapid,radial	3.36	27.17
LSSB-3	White	Greyish white	Suppressed, uniform, cottony	Radial	4.28	23.00
LSSB-4	White	Greyish white	Raised, uniform, cottony	Radial	2.48	23.11
LSSB-5	White	Greyish black	Suppressed, uniform cottony	Radial	3.84	21.44
LSSB-6	White	Greyish white	Fluffy, uniform	Radial	3.68	25.83
LSSB-7	White	Grey	Fluffy, uniform	Rapid, radial	3.49	22.38
LSSB-8	White	Greyish white	Suppressed, uniform	Radial	3.42	23.22
LSSB-9	White	Greyish black	Fluffy, uniform	Radial	1.15	23.44
LSSB-10	White	Greyish black	Raised, uniform, Cottony	Very Rapid,radial	2.91	27.89
LSSB-11	White	Greyish black	Fluffy, uniform	Very Rapid,radial	2.87	29.00

their morphological characters. At the initial stages of development the mycelium is of white in colour [Fig. 5(a)] showing the similar findings with Govardhan Rao *et al.* (2025) and at later stages it varies from greyish white (LSSB-3,6,8) to greyish black (LSSB-1,2,4,5,7,9,10,11) in colours. The mycelial topography were raised, fluffy and in some its suppressed (Table 4). The sporulation ranges from 1.15 × 10^5 to 4.28 × 10^5 among all the collected isolates. The growth pattern is generally rapid and radial, the virulent isolates have shown the very rapid growth such that the growth rate (mm/day) is varying from 21.44 mm/day to 29.00mm/day as the whole plate of 90 mm is covering with in three days this concerned pathogen is showing the more growth rate.

Morphological characterization

The formation of fruiting bodies starts from 12-15 days after inoculation. The shape of these fruiting bodies varies from globose to sub globose in shape [Fig. 5(c)].

Pycnidial arrangement was cantered (LSSB-1,2,6,9,11) and for rest of the other isolates they are scattered (LSSB- 3,7,8,10). Pycnidium of these isolates produce the pycnidiospores. The young spores are of hyaline, aseptate [Fig. 5(d)] and at later stages these matured spores are of ovoid to ellipsoid in shape with single septa and with longitudinal straiations [Fig. 5(e)]. Similar results were reported by the Ashokkumar *et al.* (2018) worked with *L. theobromae*, which is associated with coconut leaf blight.

Molecular characterization

The isolates which have shown the virulency during pathogenicity were taken for further molecular characterization

PCR amplification was performed by using the universal primers ITS 1nad ITS 4, for amplification of the ITS region in these four virulent isolates. These isolates that showed the amplicon size of 550bp and this

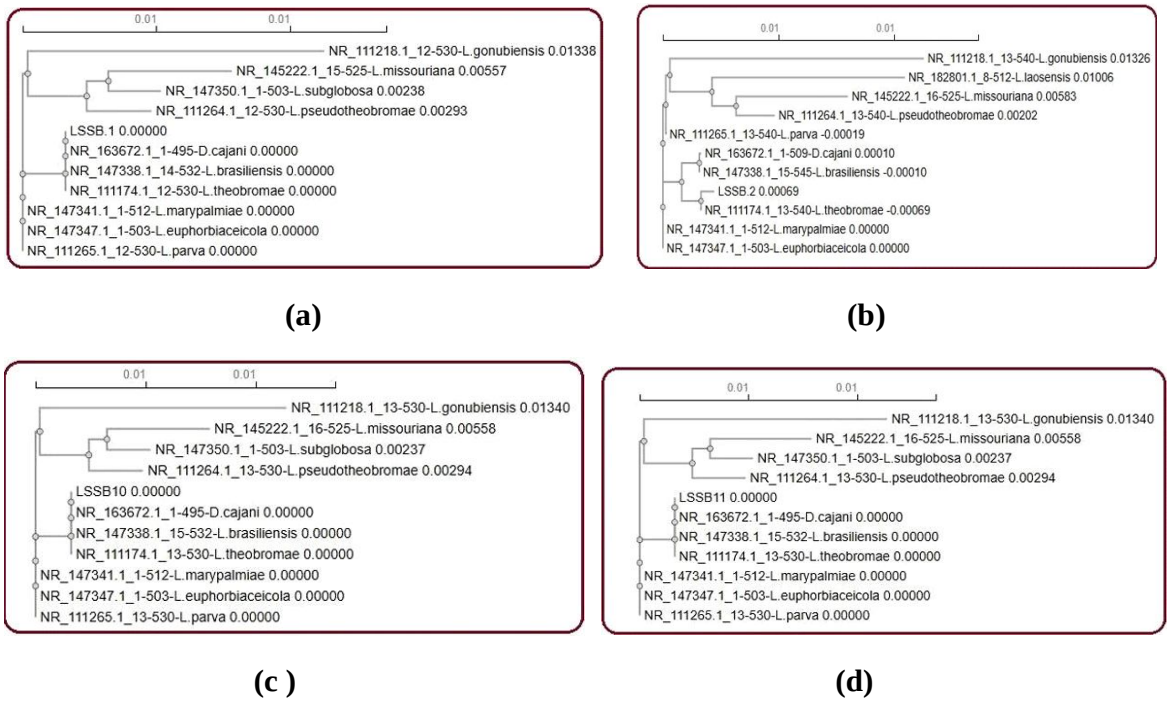


Fig. 7 : Phylogram showing the phylogenetic relationship of *Lasiodiplodia theobromae*.

Table 5 : Morphological characters.

Name of isolate	Young spores	Mature spores	Shape of spores	Fruiting body	Septation of spores	Pycinidial body formation
LLSB-1	Hyaline	Dark brown	Ovoid	Pycnidia	Single septa	At center
LSSB-2	Hyaline	Dark brown	Ellipsoid	Pycnidia	Single septa	At center
LSSB-3	Hyaline	Dark brown	Ovoid	Pycnidia	Single septa	Scattered
LSSB-4	Hyaline	Dark brown	Ellipsoid	Pycnidia	Single septa	Scattered
LSSB-5	Hyaline	Dark brown	Ellipsoid	Pycnidia	Single septa	Scattered
LSSB-6	Hyaline	Dark brown	Ellipsoid	Pycnidia	Single septa	At center
LSSB-7	Hyaline	Dark brown	Ovoid	Pycnidia	Single septa	Scattered
LSSB-8	Hyaline	Dark brown	Ellipsoid	Pycnidia	Single septa	Scattered
LSSB-9	Hyaline	Dark brown	Ellipsoid	Pycnidia	Single septa	At center
LSSB-10	Hyaline	Dark brown	Ellipsoid	Pycnidia	Single septa	Scattered
LSSB-11	Hyaline	Dark brown	Ellipsoid	Pycnidia	Single septa	At center

showing the similar findings of Ashokkumar *et al.*,2018 in 1.0% agarose gel with respect to the region of the 18S-28S rRNA intervining sequence. Then the selected virulent isolates (LSSB-1,2,10,11) were sequenced and blasted in NCBI. As it is showing the 100% homology with *L.theobromae*(Accession number QB178097, QB178098, QB178099, QB178100). Bharanideepan (2013) reported that ITS primers 1 and 4 were used to amplify.

Conclusion

The present study revealed that leaf spot and seedling blight incidence in coconut nurseries across five major coconut-growing areas of Andhra Pradesh ranged from 7.50% to 18.25%, with the highest incidence recorded at Pandirimamidi. Among the eleven isolates collected, the Palakollu isolate from West Godavari exhibited the maximum lesion diameter (28.9 mm), indicating high virulence. Variations were observed among the isolates in cultural and morphological characteristics, with radial growth rates ranging from 22.38 to 29.00 mm/day. Based on morphological, cultural, and molecular characterization, the pathogen was identified as *Lasiodiplodia theobromae*. Further studies on disease epidemiology and

management are needed to minimize disease incidence and improve coconut seedling health.

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