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Variations in Morphological and Molecular Characterization of *Lasiodiplodia theobromae* (Pat.) Griffon and Maubl Associated with Coconut Leaf Blight

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Survey was conducted for the assessment of leaf blight incidence in coconut growing districts of Tamil Nadu. The leaf blight incidence was ranged from 3.76 to 28.20 per cent in the surveyed areas and the maximum incidence was recorded in Pollachi area (28.20 per cent). Among the fifteen isolates of *Lasiodiplodia theobromae* collected, the isolate collected from Aliyarnagar (LT-CL1) produced maximum lesion size of 6.7 × 5.5 cm and it was proved to be virulence. The colony texture of the *L. theobromae* isolates varied from fluffy, suppressed to raised and the colour varied from dull white to greyish black. All the isolates showed variation in the morphological characters and the pycnidiospore size varies from 14.3 × 7.69 µm (LT-CL2) to 25.59 × 13.31 µm (LT-CL5). The *Lasiodiplodia* genus showed the amplicon size of 550bp with respect to the region of the 18S-28S rRNA intervening sequence. The fungal pathogen of leaf blight of coconut was identified as *L. theobromae* based on its morphological and molecular characters.

Key words: Coconut, Leaf blight, *Lasiodiplodia theobromae*, Morphology, Molecular characters.

Coconut (*Cocos nucifera*, L.) is a versatile crop known as “Tree of Heaven”, “Tree of Abundance”, “Tree of life” and “kalpavriksha”, as it provides food, oil, health drink, medicine, fiber, timber, fuel, and varieties of products of commercial importance. The major devastating diseases occurring in coconut in Tamil Nadu are bud rot (*Phytophthora palmivora*), Tanjore Wilt (or) basal stem end rot (*Ganoderma lucidum*), Kerala wilt (Phytoplasma), grey blight (*Pestalotiopsis palmarum*) and stem bleeding disease (*Thievolopsis paradoxa*). Among the diseases, leaf blight caused by *Lasiodiplodioa theobromae* (Pat.) Griffon and Maubl has become severe problem in different districts of Tamil Nadu like Coimbatore, Erode, Dindigul, Tirunelveli and Kanyakumari and caused yield loss of 10-25 per cent (Johnson *et al.,* 2014). The leaf blight affected coconut plants were exhibited drying of leaf lets from tip to downwards in the lower fronds and the undulated dark grey to brown colour lesions in the nuts. Ram (1989) reported that the airborne conidia *Lasiodiplodia theobromae* was the causal agent of leaf blight of coconut. Lakshmanan and Jegadeesan (2004) reported that the combination of eriophyid mite infestation and leaf blight fungus *Lasiodiplodioa theobromae* (Pat.) Griffon and Maubl caused severe damage to the coconuts during 1998 in Tamil Nadu. In Thanjavur district of Tamil Nadu, the leaf blight disease spreads at faster rate was reported by Surulirajan *et al.* (2014). *Lasiodiplodia theobromae* causing coconut rot and fall was reported in Roramia, Brazil by Haifeld-Veira and Hechet (2005). *Lasiodiplodia theobromae* (Botryosphaeriaceae: Botryosphaeriales: Dothideomycetes: Ascomycota) is cosmopolitan in nature and however, this species is more commonly found in tropical and subtropical regions (Punithalingam, 1980; Marques *et al.,* 2013). Hence, with concerning the importance of coconut leaf blight disease in different districts of Tamil Nadu, the present study was undertaken for isolation, morphological and molecular characterization of pathogen associated with coconut leaf blight disease.

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Material and Methods

Survey for Lasiodiplodia theobromae isolates

An extensive field survey was conducted during 2017 for the assessment of leaf blight incidence in the different coconut growing areas of Tamil Nadu *viz*., Coimbatore (Aliyarnagar, Pollachi, Puliyangandi, TNAU coconut farm, Thondamuthur, Negamam and Annur), Tirupur (Udumalapet, Palladam and Dharapuram), Tirunelveli (Vasudevanallur and Thenkasi), Kanyakumari (Padmanabhapuaram and Rajakkamangalam) and Krishnagiri (Paiyur) districts and samples were collected. In each selected location, average of twenty five coconut palms were selected. From each tree, twenty five leaflets in bottom ten leaves were randomly selected and scored using 0 to 5 scale for severity of disease (Johnson *et al.,* 2014). 0-5 scale (0 – No infection; 1 - < 10%; 2 – 11-25%; 3 – 26-50%; 4 – 51-75%; 5 - >75% leaf area infected). The percent disease index (PDI) was calculated based on the formula

Percent Disease Index (PDI) was worked out by using the following formula.

$$\text{Percent Disease Index (PDI)} = \frac{\text{Sum of individual ratings}}{\text{Total number of leaves observed}} \times \frac{100}{\text{Maximum grade}}$$

Pathogenicity

The diseased coconut leaf samples collected from different regions of Tamil Nadu were used for the isolation of pathogen. Infected leaf bits of (3-5 mm) were surface sterilized in 1% sodium hypochlorite solution for three minutes and washed in three changes of sterile distilled water and plated on PDA in sterilized Petri dish. Petri dishes were incubated at 28±2°C for the hyphal growth. Hyphal tip of cultures were transferred to obtain pure cultures. The pathogen was purified by hyphal tip method as described by Ricker and Ricker (1936).

Pathogenicity test was conducted by pin prick method under glass house condition. One year old coconut seedlings (Variety: ALR CN-1) were brought and the spore suspension of the pathogen were prepared from the well sporulated PDA plates contained pycnidia and spores. The pycnidial mass was macerated with sterilized pestle and mortar in water and superficial layer was taken. Later it was filtered through a cheese cloth and the spore concentration was adjusted to 5×10^5 conidia/mL by using haemocytometer (Sudha, 2007). The healthy leaves were pinpricked up to 10% of leaf area with sterile entomological pins followed by the inoculation of spore suspension (20 µL) onto the individual wounds uniformly and incubated at 28 ± 2 ºC for the symptom expression and the entire leaves were covered with polythene bags with perforations for maintaining the relative humidity. Coconut leaves inoculated with sterile water served as control.

Morphological characterization

Fifteen isolates of *L. theobromae* were grown on PDA medium to study their growth and variability in colony morphological characters. From the eight-day old culture plates, fungal disc (9 mm) was cut by a sterile corkborer and placed at the center of each sterile Petri dish (90 mm) containing PDA medium. The plates were incubated at room temperature (28±2ºC) for 7 days. Colony growth, color and texture observations were taken on 7 days after incubation. Pycnidial characters of each isolate *viz.,* days to production, arrangement and size were observed 30 days after incubation. Pycnidiospore characters such as size, color and septation were also recorded.

Molecular characterization

DNA isolation

The seven days old culture of the pathogen disc was transferred into 250 mL Erlenmeyer flasks containing 100 mL potato dextrose broth (PDB) and incubated at room temperature for 7 days. Mycelium was harvested by filtration through sterile filter and stored at -80ºC until used for DNA extraction. To extract the DNA, one gram of frozen mycelium was ground into fine powder in liquid nitrogen and incubated in 5 mL of 2 % CTAB extraction buffer [10 mM trisbase (pH 8.0), 20 mM EDTA (pH 8.0), 1.4 M NaCl, CTAB (2 %), mercaptoethanol (0.1%) and PVP (0.2 %)] at 65 ºC for 15 minutes. The suspension was added with equal volume of phenol-chloroform-isoamylalcohol (25:24:1) mixture. It was vortexed to mix two phases followed by a centrifuge at 12,000 rpm for 5 minutes. The supernatant was transferred to clean tube and mixed with equal volume of ice cold isopropanol. It was incubated at 25˚C for DNA precipitation. The precipitate was collected by centrifugation and the pellet was washed with 0.1M Ammonium acetate in 70% ethanol and incubated for 15 minutes. The pellet was resuspended in 500 µL TE buffer (10 mM Tris, 1 mM EDTA, pH 8.0) and the DNA concentration was estimated spectrophotometrically (Sangeetha *et al.,* 2012).

PCR amplification and sequencing

The universal primers ITS 1 (Forward) and ITS 4 (Reverse) were used to amplify the ITS regions of *L. theobromae* described by White *et al*. (1990).

ITS 1 - 5'-CTTGGTCATTTAGAGGAAGTAA-3'

ITS 4 - 5'-TCCTCCGCTTATTGATATGC-3'

PCR reaction mixture consisted of 10 µL of 2x PCR master mix, 1 µL of forward primer and 1 µL of reverse primer, template DNA 2 µL and 5 µL of sterile water. The amplification was carried out in a thermocycler. PCR reaction was performed with initial denaturation step at 94ºC for 5 min, 35 cycles of amplification (20 seconds for denaturation at 94ºC, 30 seconds for primer annealing at 55ºC and 30 seconds for extension at 72ºC) and one cycle of final extension at 72ºC for 5 minutes was performed in thermocycler (EppendorfMastercycler, Westbury, New York).The amplified PCR products were run on 1.5% agarose gel in Tris-acetate buffer. The gel was stained with ethidium bromide, visualized on an UV-transilluminator and photographed in the gel documentation unit (Alpha Innotech Corp, USA). The sizes of the PCR products were determined by comparison with standard 100 bp molecular marker.

The data obtained were statistically analyzed (Gomez and Gomez, 1984) and the treatment means were compared by Duncan' package used for analysis was IRRISTAT version 92.0 developed by the International Rice Research Institute, Biometrics Unit, The Philippines.

Results and Discussion

Survey was conducted in a randomized manner during 2017 in different districts of Tamil Nadu to record the prevalence of coconut leaf blight. The coconut palms having leaf blight symptoms were selected from Coimbatore (Aliyarnagar, Pollachi, Puliyangandi, TNAU Orchard, Thondamuthur, Negamam and Annur), Tirupur (Udumalapet, Palladam and Dharapuram), Tirunelveli (Vasudevanallur and Thenkasi), Kanyakumari (Padmanabhapuaram and Rajakkamangalam) and Krishnagiri (Paiyur) and the disease index was calculated by Johnson *et al.*

(2014). Coconut leaf blight incidence was recorded in all the areas surveyed and the incidence ranged from 3.76 to 28.2 per cent. The maximum leaf blight incidence was recorded in Pollachi (28.21 per cent) was on par with Aliyarnagar (27.58 per cent) followed by Puliyangandi which has incidence of 25.91 per cent, while the minimum incidence was recorded in Thenkasi (3.76 per cent) (Table 1).

Table 1. Survey and collection of *Lasiodiplodia theobromae* isolates

District	Place of collection	Co-ordinates		Altitude (MSL)	PDI*
		Latitude (o N)	Longitude (o E)		
Coimbatore	Aliyarnagar	10.4881	76.9657	260.0	27.58a
	Pollachi	10.6572	77.0106	287.65	28.21a
	Puliyangandi	10.4888	76.9766	310.57	25.91b
	TNAU coconut farm	11.0123	76.9355	426.40	22.13c
	Thondamuthur	10.9880	76.8459	450.00	25.17b
	Negamam	10.7426	77.1032	360.13	17.58d
	Annur	11.2321	77.1067	379.06	12.84g
	Udumalapet	10.5845	77.2514	364.68	21.56c
Tirupur	Palladam	10.9996	77.2807	374.03	17.82d
	Dharapuram	10.7329	77.5218	261.66	14.24f
Tirunelveli	Vasudevanallur	9.2395	77.4113	183.44	5.68h
	Thenkasi	8.9590	77.3129	163.30	3.76i
Kanyakumari	Padmanabhapuram	8.2425	77.3152	47.03	15.46e
	Rajakkamangalam	8.1290	77.3640	17.99	13.72fg
Krishnagiri	Paiyur	12.3696	78.2193	36.87	22.07c

*Mean of three replications.

In a column, means followed by a common letter are not significantly different at the 5% level by DMRT.

The incidence of leaf blight was reported by several workers. Leaf blight incidence upto 27.04 per cent was recorded in Manakadavu area of Pollachi in Coimbatore district (Athira, 2017). Incidence of *L. theobromae* on nuts causing nut rot upto 22.0 per cent was recorded in Thondamuthur of Coimbatore district (Dheepa *et al.,* 2018). Prevalence of coconut leaf blight has been reported recently from different areas of Tamil Nadu viz., Coimbatore, Dindigul, Erode, Tirunelveli and Kanyakumari districts (Johnson *et al.,* 2014) and it indicated that leaf blight emerging as a serious one in coconut growing areas of Tamil Nadu.

All the fifteen isolates of *L. theobromae* were artificially inoculated on coconut seedlings and observed for their virulence activity. The results of the pathogenicity test indicated that, among the all isolate the isolate LT-CL1 collected from Aliyarnagar produced maximum lesion length of 6.7 cm followed by Pollachi (LT-CL2) isolate (6.3 cm lesion size) and was on par with following isolates Udumalapet (LT-CL8) and Dharapuram (LT-CL10) which produced (6.1 cm and 6.2 cm lesion size) compared to control. The isolate collected from Rajakkamangalam (LT-CL14) produced minimum lesion length of 4.1 cm (Table 2). The pathogenicity test for *L. theobromae* was reported by several scientists. Surulirajan *et al.* (2004) reported that coconut seedlings inoculated with the mycelial disc of *L. theobromae* exhibited straw coloured lesions on the inoculated areas at 7 days after inoculation.

Table 2. Pathogenicity test for *L. theobromae* isolates on coconut seedlings

Isolates No.	Place of collection	Lesion size (cm)*	
		Length	Breadth
Lt-CL1	Aliyarnagar	6.7^{a}	5.5^{a}
Lt-CL2	Pollachi	6.3^{b}	5.2^{b}
Lt-CL3	Puliyangandi	5.5^{fg}	4.3^{e}
Lt-CL4	TNAU coconut farm	6.0^{cd}	3.5^{h}
Lt-CL5	Thondamuthur	5.7^{ef}	3.9^{f}
Lt-CL6	Negamam	4.8^{h}	3.5^{h}
Lt-CL7	Annur	5.6^{ef}	4.0^{f}
Lt-CL8	Udumalapet	6.1^{bc}	4.2^{e}
Lt-CL9	Palladam	5.5^{fg}	4.8^{c}
Lt-CL10	Dharapuram	6.2^{bc}	4.4^{e}
Lt-CL11	Vasudevanallur	5.3^{g}	3.9^{f}
Lt-CL12	Thenkasi	4.3^{i}	3.7^{g}
Lt-CL13	Padmanabhapuram	5.8^{de}	4.6^{d}
Lt-CL14	Rajakkamangalam	4.1^{i}	3.4^{h}
Lt-CL15	Paiyur	5.8^{de}	5.2^{b}
Control		0.0^{k}	0.0^{i}

*Mean of three replications.; In a column, means followed by a common letter are not significantly different at the 5% level by DMRT.

Dheepa *et al.* (2018) carried out pathogenicity test on nuts by following pin prick method and

the inoculated nuts were exhibited brown lesions, undulated wavy margins to cracking symptoms with black pycnidia.

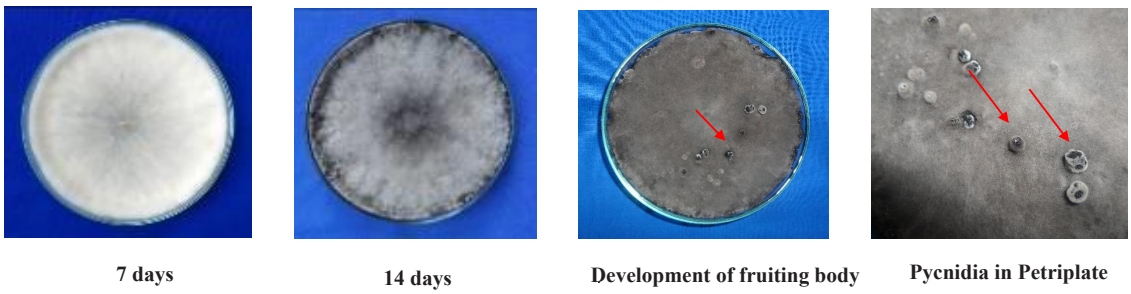
The *L. theobromae* isolates were grown in PDA medium to study their morphological characters. Colonies colour of the *L. theobromae* isolates varied from dull white (LT-CL1, LT-CL14), white (LT-CL15), cottony to grey (LT-CL3, LT-CL5 and LT-CL7), greyish black (LT-CL2, LT-CL4 and LT-CL9) and greyish white (LT-CL6, LT-CL10 and LT-CL12).

Table 3. Morphological characters of *L. theobromae* isolates

Isolate No.	Colony texture and colour	Pycnidia			Pycnidiospore			
		Days for production	Arrangement	Diameter (µm)	Days for production	Colour	Size (µm)*	
							Length	Width
Lt-CL1	Fluffy, uniform, dull white in colour	23	Scattered	118-184	28	Dark brown	17.98^{f}	11.20^{e}
Lt-CL2	Raised, irregular, cottony white changing to greyish black	21	Periphery	93-179	26	Light brown	14.30^{h}	7.69^{f}
Lt-CL3	Fluffy, uniform, white changing to grey colour	25	Scattered	125-208	29	Dark brown	23.26^{b}	13.31^{b}
Lt-CL4	Suppressed, irregular white changing to greyish black	24	Scattered	115-176	29	Dark brown	21.64^{c}	11.44^{e}
Lt-CL5	Raised, irregular, cottony changing to grey colour	22	Scattered	108-178	27	Dark brown	25.59^{a}	13.90^{a}
Lt-CL6	Fluffy, uniform, greyish white in colour	23	Scattered	99-196	28	Dark brown	22.89^{b}	12.18^{d}
Lt-CL7	Raised, irregular, cottony changing to grey colour	25	Scattered	124-201	30	Dark brown	20.52^{d}	12.20^{d}
Lt-CL8	Raised, uniform, cottony changing to grey colour	24	Scattered	118-186	28	Light brown	20.27^{d}	12.68^{cd}
Lt-CL9	Suppressed uniform, fluffy white changing to greyish black	23	Centered	96-176	27	Dark brown	18.09^{f}	13.11^{bc}
Lt-CL10	Raised, irregular, cottony greyish white in colour	24	Scattered	106-188	29	Dark brown	17.24^{g}	11.33^{e}
Lt-CL11	Suppressed, uniform, cottony white changing to black	23	Scattered	110-192	27	Dark brown	19.22^{e}	11.43^{e}
Lt-CL12	Suppressed, uniform, cottony greyish white in colour	23	Scattered	118-202	27	Dark brown	19.30^{e}	12.70^{cd}
Lt-CL13	Raised, irregular, cottony changing to black	25	Scattered	125-203	29	Dark brown	20.95^{cd}	12.76^{bcd}
Lt-CL14	Fluffy, uniform, dull white in colour	24	Centered	86-182	28	Dark brown	18.77^{e}	11.26^{e}
Lt-CL15	Raised, irregular white in colour	26	Scattered	98-184	31	Dark brown	21.41^{c}	12.33^{d}

*Mean of three replications.

In a column, means followed by a common letter are not significantly different at the 5% level by DMRT.

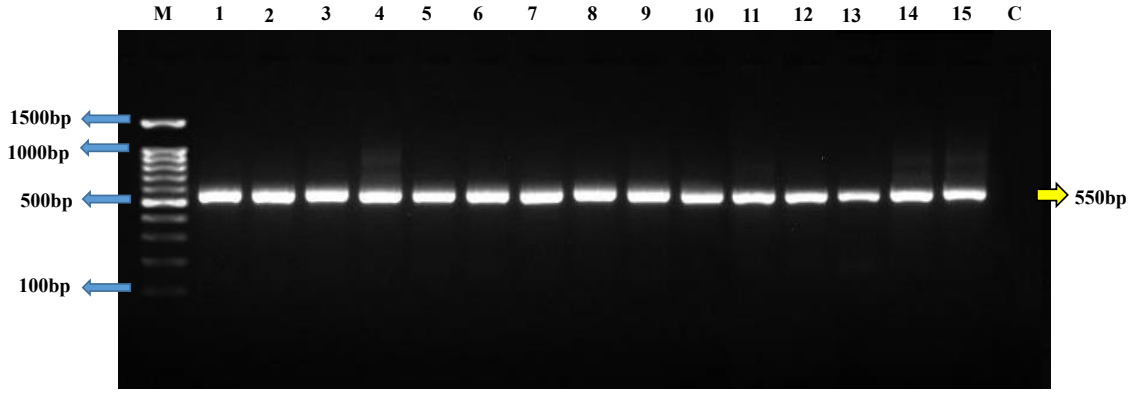


7 days | 14 days | Development of fruiting body | Pycnidia in Petriplate

Releasing of spores from pycnidia (40x) | Immature conidia (40x) | Matured conidia (40x)

Fig 1. Cultural and pycnidial characters of *L. theobromae* isolates

The mycelial topography were raised, fluffy and in some isolates it was suppressed. The time taken for pycnidial production varied from 21 days (LT-CL2) to 26 days (LT-CL15) in PDA medium. Pycnidial arrangement was periphery (LT CL2 and LT-CL7), centered (LT-CL9 and LT-CL14) and rest of isolates were in scattered manner. Pycnidia size varied from 93-208 µm in diameter. Pycnidium of *L. theobromae* produced pycnidiospores and the spores were initially hyaline, elliptical and aseptate and later turned to dark brown coloured, bicelled conidia with longitudinal striations on the outer wall of the spore. The size of pycnidiospores ranged from 14.30 µm to 25.59 µm in length and 7.69 µm to 13.90 µm in width. All the



M: Ladder 100 bp
1: LT-CL1
2: LT-CL2
3: LT-CL3
4: LT-CL4
5: LT-CL5
6: LT-CL6
7: LT-CL7
8: LT-CL8
9: LT-CL9
10: LT-CL10
11: LT-CL11
12: LT-CL12
13: LT-CL13
14: LT-CL14
15: LT-CL15
C: Control

Fig 2. PCR amplification of rRNA-ITS region of the isolates of *L. theobromae*

isolates significantly varied in the size of the pycnidiospores. The maximum length of pycnidiospore produced in LT-CL5 isolate (25.59 µm) followed by isolates LT-CL3 (23.26 µm) and LT-CL6 (22.89 µm) was on par with each other. The minimum length was observed in LT-CL2 isolate (14.30 µm) (Table 3). Similar results were also reported by Sathya *et al.* (2017) worked with *L. theobromae* and the colony colour varied from greyish white to greyish black with abundant sporulation occurred. The colonies are greyish black, cottony white to greyish white and produced fluffy mycelium. The conidia was initially hyaline, aseptate become dark brown, one sepatate and size were 20.2 to 25.3 µm × 10.3 to 13.90 µm (Dheepa *et al.,* 2018).

PCR amplification of ITS region in the fifteen isolates of *Lasiodiplodia* was performed using the universal primers ITS 1 and ITS 4. The *Lasiodiplodia* genus showed the amplicon size of 550bp in 1.0% agarose gel with respect to the region of the 18S-28S rRNA intervening sequence for *Lasiodiplodia* sp (Fig. 2). Then the selected virulent isolates (LT-CL1 and LT-CL2) were sequenced and blasted in NCBI. It showed 99 % homology with *L. theobromae* (accession number MG768931 and MG768942 respectively. Hence, the pathogen was identified as *L. theobromae*. Bharanideepan (2013) reported that the ITS primers 1 and 4 have been used to amplify the genus-specific PCR assay for the rapid identification in different isolates of *L. theobromae*. The identification of *L. theobromae* through amplifying of ITS regions with forward and reverse primer pairs was reported by (Al-Hammadi *et al.,* 2018 and Munirah *et al.,* 2017).

Conclusion

The leaf blight pathogen of coconut was identified as *L. theobromae* based on its cultural, morphological and molecular characteristics from this present study. Though all the isolates showed variation in their morphological characters, PCR amplification of the isolates revealed that all isolates were amplified at 550 bp. Hence, it was proved that all the isolates were belongs to *L. theobromae.* Further investigation has to be carry out on the epidemiology studies and the disease management practices of leaf blight under field condition.

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References

Al-Hammadi, M. S., Al-Shariqi, R., Maharachchikumbura, S. S. N. and A. M. Al-Sadi. 2018. Molecular identification of fungal pathogens associated with date palmroot diseases in the United Arab Emirates. *Journal of Plant Pathology,* https://doi.org/10.1007/s42161-018-0089-8.

Athira, K. 2017. Survey, identification and estimation of damage in major diseases of coconut. *International Journal of Current Microbiology and Applied Sciences*, **6(12)**: 416-423.

Bharanideepan, A. 2013. Studies on die-back of acid lime (*Lasiodiplodia theobromae*) in acid lime. M. Sc. (Ag) Thesis, Tamil Nadu Agricultural University. Coimbatore. p: 45.

Dheepa, R., Goplakrishnan, C., Kamalakannan, A., Nakkeeran, S., Mahalingam, C. A. and J. Suresh. 2018. Coconut nut rot disease in India: Prevalence, characterization of pathogen and standardization of inoculation techniques. *International Journal of Current Microbiology and Applied Sciences,* **7(2)**: 2046-2057.

Gomez, K. A. and A. A. Gomez. 1984. Statistical procedures for agricultural research. (2-Ed.) A Wiley Interscience Publication, John Wiley and Sons. New York. p: 207.

Halfeld-Veira, B. A. and K. L. Nechet. 2005. Queda de Frutos m Coqueiro causada por *Lasiodiplodia theobromae* em Roraima. *Fitopatologia Brasileira,* **30**: 203.

Johnson, I., Meena, B. and K. Rajamanickam. 2014. Biological management of leaf blight disease of coconut using rhizosphere microbes. *Journal of Plantation Crops*, **42(3)**: 364-369.

Lakshmanan, P. and R. Jegadeesan. 2004. Malformation and cracking of nuts in coconut palms (*Cocos nucifera*) due to the interaction of the eriophyid mite *Aceria guerreronis* and *Botryodiplodia theobromae* in Tamil Nadu, India. *Journal of Plant Diseases and Protection,* **111(2)**: 206-207.

Marques, M. W., Lima, N. B., de Morais, M. A., Barbosa, M. A., Michereff, S. J. and M. P. Camara. 2013. Species of *Lasiodiplodia* associated with mango in Brazil. *Fungal Diversity,* **61**: 181-193.

Munirah, M. S., Azmi, A. R., Yong, S. Y. C. and M. Z. Nur Ain Izzati. 2017. Characterization of *Lasiodiplodia theobromae* and *L. pseudotheobromae* causing fruit rot on pre-harvest mango in Malaysia. *Plant Pathology and Quarantine,* **7(2)**: 202-213.

Punithalingam, E. 1980. Plant diseases attributed to *Botryodiplodia theobromae* Pat. In: Bibliotheca Mycologica Series, No. 71, J. Cramer, Vaduz. p: 121.

Ram, C. 1989. Epidemiologia e controle quimico da queima-das-folhas (*Botryodiplodia theobromae*) do coqueiro (*Cocos nucifera* L.). *Fitopatologia Brasileira,* **14(3)**: 215-220.

Ricker, A. J. and R. S. Ricker. 1936. Introduction to research on plant diseases. Jon. S. Swift Co. Inc., St. Lovis, Chicago, New York. p: 117.

Sangeetha, G., Anandan, A. and S. U. Rani. 2012. Morphological and molecular characterization of *Lasiodiplodia theobromae* from various banana cultivars causing crown rot disease in fruits. *Archives of Phytopathology and Plant Protection*, **45(4)**: 475-486.

Sathya, K., Parthasarathy, S., Thiribhuvanamala, G. and K. Prabakar. 2017. Morphological and molecular variability of *Lasiodiplodia theobromae* causing stem end rot of mango in Tamil Nadu, India. *International Journal of Pure and Applied Biosciences*, **5(6)**: 1024-1031.

Sudha, A. 2007. Molecular characterization and pathogenic variability of the isolates of *Botryodiplodia theobromae* (Pat.) associated with eriophyid mite *Aceria guerreronis* Keifer. infestation on coconut and their management. Ph.D. Thesis, Tamil Nadu Agricultural University, Coimbatore. p: 37.

Surulirajan, M., Rajappan, K., Satheesh, K., Annadurai, K., Jeevan, K. and M. Asokhan. 2014. Isolation of leaf blight of coconut and screening of fungicides against the pathogen. *International Journal of Tropical Agriculture,* **32(3)**: 475-478.

White, T. J., Bruns, T., Lee, S. and J. L. Taylor. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: PCR protocols: A guide to methods and applications. M.A. Innis, D.H. Gelfand, J.J. Sninsky and T.J. White, (Eds.), Academic press, San Diego. p: 315-322.

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