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NEW REPORT OF LEAF SPOT DISEASE OF *CLERODENDRUM INDICUM* CAUSED BY *CURVULARIA LUNATA*

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ABSTRACT

This is a maiden report of necrotic leaf spot disease of *Clerodendrum indicum*, an important medicinal plant, caused by fungal pathogen *Curvularia lunata*. *Clerodendrum indicum* (L.) Kuntze, as an important medicinal plant is grown extensively in India, Nepal, China, Japan, Srilanka, West Indies etc. and considered for its potency against asthma, cough, fever, intestinal worms, arthritis etc. The fungal pathogen was isolated from the diseased plant tissue and pure culture was being established. It has been identified as *Curvularia lunata* The plant is native in India and is found throughout south eastern Asia. This plant is used to treat cough, asthma, bronchitis and other respiratory diseases. Morphological study, pathogenicity test , and 18s rDNA sequencing confirmed the pathogen *Curvularia lunata*.

KEYWORDS: ***Clerodendrum indicum*, leaf spot disease, 18S rDNA gene Sequencing, Phylogenetic tree, *Curvularia lunata*.**

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INTRODUCTION

Clerodendrum indicum (L.) Kuntze, a woody herbaceous, perennial plant belonging to the family Verbenaceae, is a native of India, commonly known as Bamonhati and widely used against asthma, cough, fever, etc.[1] . This plant originated in eastern India and found dispersed throughout south eastern Asia, India, Nepal, Bhutan, Sri Lanka, and Southern China. In India, it is found mainly in West Bengal and Assam [2].

MATERIALS AND METHODS

PLANT MATERIAL

Healthy and young*Clerodendrum indicum* plants were collected for research experiment from Hatgobindopur college, Burdwan, West Bengal, India.

METHODS

(a) Study of Disease Symptoms

The symptoms of necrotic leaf spot disease were observed on the leaves of the plant, *Clerodendrum indicum.*The diseased plants were collected and the voucher specimens were deposited in the herbarium of plant pathology unit of the department of Botany, The University of Burdwan, West Bengal, India. The symptoms first appered on the upper surface of the leaf, especially on margin and the apex ,laterspread gradually to the whole leaf extending up to the petiole.In the early stage, symptom showed up i as yellowish-brown spot and later it turned dark brown to grayish-brown surrounded by a yellow halo. The spots were 1 cm to 1.5 cm in diameter. Symptoms were more common on mature leaves than immature ones. Severe condition of disease were marked by heavy defoliation. (Fig 1).

(b) Isolation of Pathogen

For isolation of the pathogen, small leaf pieces (5 mm in diameter) were excised from disease infected region of the leaf and washed with sterilized distilled water followed by detergent Tween -20. Then it was washed serially in 70% ethanol for 1 min, 0.1% $HgCl_2$ for 1 min followed by 1% NaOCl solution for 30 sec. Finally leaf pieces were washed with sterilized double distilled water (MiliQ) and transferred into potato dextrose agar medium (pH 6.2), incubated at 30±1˚C for 7 days. A velvety brownish–black colored fungal colony was developed in culture which was then sub-cultured in fresh PDA medium. The pathogenecity test was done and confirmed by Koch's postulates

(c) Studies in Scanning Electron Microscopy

The fungus was studies in a phase contrast microscope (Leica DMLB 3000) (Fig 2-6). Slides were prepared using the culture of the pathogen (sample mounted in 3% KOH solution) and used for scanning electron microscopic study following the method as adopted by Correl *et al.*[3] .Scanning electron microscopic study was done in a Scanning Electron Microscope (SEM Hitachi S-530, 15 KV) (Fig 7 & 8).

(d) Isolation of DNA for 18S rDNA gene Sequencing

Fungal DNA was isolated following CTAB method using CTAB (Cetyltrimethyl Ammonium Bromide) buffer with 0.1% ß-marcaptoethanol. Mycelia mat of two week old culture was crushed in CTAB buffer and centrifuge at 10,000 rpm for 10 min. Supernatant was taken and discard the pellet. Supernatant was mixed with Chloroform: Isoamyl alcohol(24:1) and store at 4˚C for 24 hours. DNA was precipitated and the supernatant was discarded, the pellet was mixed with 70% ethanol and centrifuge at 10,000 rpm for 10 min. After that, the supernatant was being discarded and DNA was air dried. The DNA was then dissolved in 100µL of TE buffer (Tris-EDTA buffer). The DNA sample was then used for 18S rDNA sequencing. Subsequent BLAST analysis and phylogenetic tree was made.

RESULTS AND DISCUSSION

(a) Scanning Electron Microscopic Studies

Under SEM studies, terminal and intercalary conidia were observed which were found to be developed from septate melanized thick walled branched hyphe. Conidia were dark brown in colour and septate transversely from edge to edge of the conidial wall. All conidia were crescent shape, curved, 3-4 celled, middle cell of mature conidium is larger than the others and contains prominent oil globule. On the basis of growth characteristics, colony morphology, light and scanning electron microscopic studies of the pathogen was identified to be a species *Curvularia* .

(b) Identification of Pathogen

The culture of the pathogen was then sent to the Microbial Type Cultural Collection and Identification Centre, Division of Plant Pathology, Indian Agricultural Research Institute (IARI), New Delhi for authentic identification and has been identified as *Curvularia lunata* (ID No. 7982.10). The pathogen has been deposited in the Microbial Type Culture Collection Centre (MTCC) of IARI, New Delhi bearing the ID No. 7982.10 and is now being maintained as voucher specimen in the Mycology and Plant pathology laboratory of Botany Department, The University of Burdwan. Finally, 18S rDNA gene sequencing of the pathogen by sanger methods and the phylogenetic tree was prepared using the software phylogeny..fr.

(c) Sequencing of 18S rDNA gene of the pathogen

The ribosomal I gene sequencing (598 bp) of the pathogen was done by ITS1-5.8S-ITS2 region. PCR primers- (for ITS1-forward sequence-5′-TCCGTAGGTGAACCTGCGG-3′ and for ITS2-5′TCCTCCGCTTATTGATATGC-3′).The details of the 18S rDNA gene sequence was; internal transcribed spacer1, 5.8S ribosomal RNA gene, and internal transcribed spacer2, complete sequence; and 28S ribosomal RNA gene partial sequence.)

>ani
TCCGTAGGTGAACCTGCGGAGGGATCATTACACTATAAAATATGAAGGCTGTACGCGGCTGTGCTCTCGG
GCCAGTTTTGCGGAGGCTGAATTATTTATTACCCTTGTCTTTTGCGCACTTGTTGTTTCCTGGGCGGGTT
CGCCCGCCACCAGGACCACATCATAAACCTTTTTTATGCAGTTGCAATCAGCGTCAGTATAACAAATGTA
AATCATTTACAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATA
CGTAGTGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCTTTGGTATTCCA
AAGGGCATGCCTGTTCGAGCGTCATTTGTACCCTCAAGCTTTGCTTGGTGTTGGGCGTTTTTTGTCTTTG
GTTGCCAAAGACTCGCCTTAAAAGGATTGGCAGCCGGCCTACTGGTTTCGCAGCGCAGCACATTTTTGCG
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GATACCCGCTGAACTTAAGCATATCAATAAGCGGAGGA

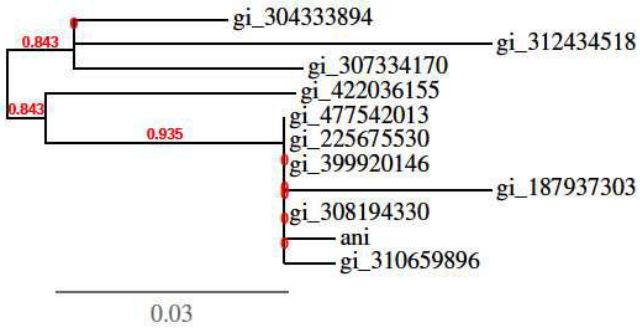

Figure A

Phylogenetic Tree and dendrogram of sequence to show the homology of "ani" sequence
Phylogenetic tree based on 18SrDNA gene sequence analysis, showed the relationship of strain Curvularia lunata (ID No. 7982.10) with other Curvularia lunata strains. Numbers of the node indicate bootstrap value. The tree was generated using "phylogeny.fr" software, Bar 0.03 substitutions per site. http://blast.ncbi.nlm.nih.gov/

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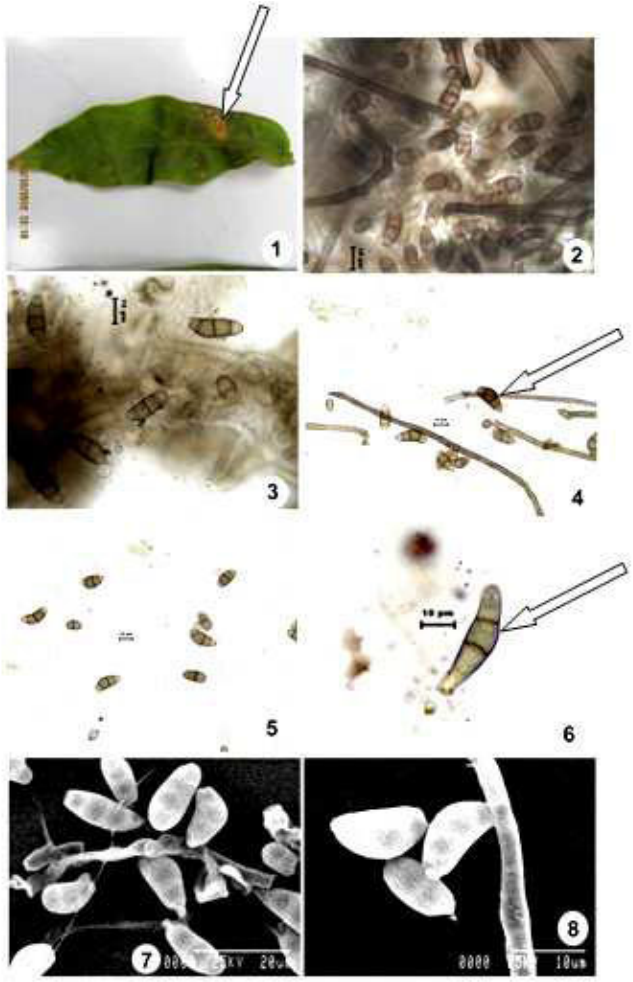

B. Figures showing the disease symptoms and its causal organism
1. A leaf showing symptoms; 2. Conidia under phase contrast microscopic field; 3. Mature conidia under phase contrast microscopic field; 4. Mature conidia cut from the tip of conidiophores; 5. Mature isolated conidium just prior to germination; 6. A mature germinating conidium; 7. A number of conidia under SEM; 8. Conidia attached to the conidiophores under SEM.

CONCLUSION

To the best of our knowledge, this is the first report of leaf spot disease of *Clerodendrum indicum* caused by *Curvularia lunata*. Understanding the mechanism of the pathogenesis is required to get more comprehensive knowledge that will help us to know about diseases of medicinal plants. This will help us to take serious scientific measures for protection and conservation of little-explored medicinal plants like *Clerodendrum indicum.* At present, analysis of the gene sequence data and strategies on plant protection of this plant is in progress.. The present communication envisages only on the disease report of this valuable medicinal plant.

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