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Pathrachur (*Coleus barbatus*) a new host record for *Diaporthe tulliensis* causing leaf spotin Bihar, India

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Coleus barbatus (local name patharchur) is an important medicinal plant of the Lamiaceae. It has been in traditional Ayurvedic medicine for curing various liver diseases, glaucoma, asthama, disorders and this is the only sources of diterpenois forskoin. Despite its medicinal value, plant disease affecting *Coleus barbatus* received little in India. *Coleus barbatus* is attacked by various pathogens but out of them leaf spot is major disease.During fixed plot survey from 2021-2024,a new disease was observed on patharchur in Bihar, India. The causal organism was identified as *Diaporthe tulliensis*R.G. Shivas, Vawdrey & Y.P. Tan based on its morphological and molecular characterization.

The symptom of disease stared in month of July-August with the appearance of small irregular shaped spots on the leaves of the plant. Spots were initially light to dark brown and surrounded by chlorotic yellow halo which gradually spread to the leaf centre followed by yellowing drying and dropping of the whole leaf. The number of spots and disease severity was highest in the month of October.

Keywords : *Coleus barbatus, Diaporthe tulliensis,* leaf spot

INTRODUCTION

Patharchur (*Coleus barbatus*) also known by the synonyms *Plectranthus barbatus* and incorrectly *Coleus forskalaei* is a tropical perennial medicinal plant. The plant root tubers eaten as a condiment in India and some *Coleus* spp. are used in traditional Ayurvedic medicines. Herbal teas made from *C. barbatus* contain rosmarinic acid and also flavonoid glucuronides and diterpenoids (Fale *et al.*2009; Porfirio *et al.*2010). The tubers of *C. barbatus* contain forskolin useful for pharmaceutical preparations and research in cell biology (Alasbahi *et al.*2010). During fixed plot survey, a new disease was observed on patharchur and symptoms of disease and morphological characteristics of pathogen were studied. Therefore the main aim of the study was to identify causal organism of leaf spot of *Coleus barbatus* for further study.

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MATERIALS AND METHODS

The present investigation was conducted in the Biocontrol Lab, Dr. Rajendra Prasad Central Agricultural, University, Pusa, Samastipur, Bihar, India.

Study of symptoms of disease

During growing season of 2021-24 aleaf spot consistently was observed on patharchur in the experimental field of All India Co-ordinated Research Project on Medicinal and Aromatic Plants, Dr. Rajendra Prasad Central Agricultural, University, Pusa, Samastipur (25.9845° N, 85.6743° E), Bihar, India, with the disease incidence was 20-80 % in fixed plot. Infected leaves were collected from the from the field and spots on patharchur were carefully examined.

Isolation of pathogen

The fungus was isolated from leaves exhibited spot symptom in the plant. To identify causal agent, lesions (5-7 mm in diameter) were cut from infected leaves and surface sterilized prior to isolation of pathogens from contaminated plant tissues 0.1% sodium hypochlorite for 1 min and then washed thrice with sterilised distilled water. The samples were air dried and placed in Petri plates containing PDA (Potato Dextrose Agar) media and incubated at 28±1ºC in a biological oxygen demand (BOD) incubator to promote the pathogen growth. White mycelium growth started growing in 3 days and mycelium further transferred to PDA slants to obtain pure culture. Sub culturing of the isolated pathogen were made at 15 days interval.

Morphological and Molecular Characterization

The slide of fungal cultural was prepared to study the morphology such as the characteristics of the hyphae, conidiophores, spores etc. The prepared slides were observed under compound microscope. For molecular characterization and species level identification, pure culture of fungus wassent to National Fungal Cultural Collection of India (NFCCI), Agharkar Research Institute, Pune, Maharashtra, India.

Pathogenicity test

Pathogenicity test was done on five healthy pathrachur leaves using pin prick inoculation with 10 μl spore suspension (1x10^{6}spores/ml). Six days after inoculation *D. tulliensis* isolate caused necrotic spots and leaf blight on leaves. Diseased leaves turned from light to dark brown. Untreated control showed no symptoms. *D. tulliensis* was re-isolated from diseased leaves, fulfilling Koch's postulates.

RESULTS AND DISCUSSION

Study of symptoms and pathogenicity

The typical symptom formed initially on leaves of the plant as brownish spot that were irregular in shape and size, light to dark brown and surrounded by a chlorotic yellow which gradually spread to the leaf centre followed by yellowing drying and dropping of the whole leaf (Fig.1).Pathogenicity test was done on five healthy pathrachur leaves using pin prick inoculation with 10 μl spore suspension (1x10^{6}spores/ml). Six days after inoculation *D. tulliensis* isolate caused necrotic spots and leaf blight on leaves. Diseased leaves turned from light to dark brown. Untreated control showed no symptoms. *D. tulliensis* was re isolated from diseased leaves, fulfilling Koch's postulates.

Morphological and molecular identification of pathogen

Colonies on PDA at 28±2ºC after 7th days, fast growing, floccose dull yellow with yellowish exudate, reverse dark ruby. Hyphae branched, septate, thick and thin, hyaline to light olivaceous. Thick hyphae constricted near septa (5.25 ìm wide), thin hyphae upto 1.0 ìm wide, anastomoses (Fig.1).

On the basis of morphological characteristics, the fungus was identified as *Diaporthe tulliensis* (Shabong and Kayang, 2023). The pure culture of isolate was sent to National Fungal Cultural Collection of India (NFCCI), Agharkar Research Institute, Pune, Maharashtra, India for morphological and molecular identification. Accession no. NFCCI 5354 and Genbank Accession no. OQ831035obtained byNational Fungal Cultural Collection of India (NFCCI), Agharkar Research Institute, Pune, Maharashtra, India and National Centre for Biotechnology Information(NCBI) USA respectively.

Identification was confirmed by amplification of the internal transcribed spacer(ITS) region. Fungal DNA from isolate was amplified by PCR using primers of ITS (White *et al.* 1990). A BLASTn analysis of these sequences showed 100% identity with reference strain RST-3 of *Diaporthe tulliensis* R.G. Shivas, Vawdrey & Y.P. Tan**.** in the ITS (MN911384), RST-2(MN911383), RST-1(MN911382), KFRD-49 (KX866889), J35S (ON920154), J34S (ON920153), N33S (ON920152), N32S (ON920151) and M29S (ON920150). Phylogenetic analyses were done using the Maximum Likelihood method based on concatenated data set and reference CBS strains using MEGA 11 software (Tamura *et al.* 2021). The isolate clustered in the same group as *D. tulliensis* (Fig.2),thus confirming the

Fig. 1: Leaf spot symptoms on Patharchur infected by *Diaporthe tulliensis*, (a) Healthy plants (b) diseased plants (c) Cultural growth of *D. tulliensis* (d) Septated branched hyphae of the fungus.

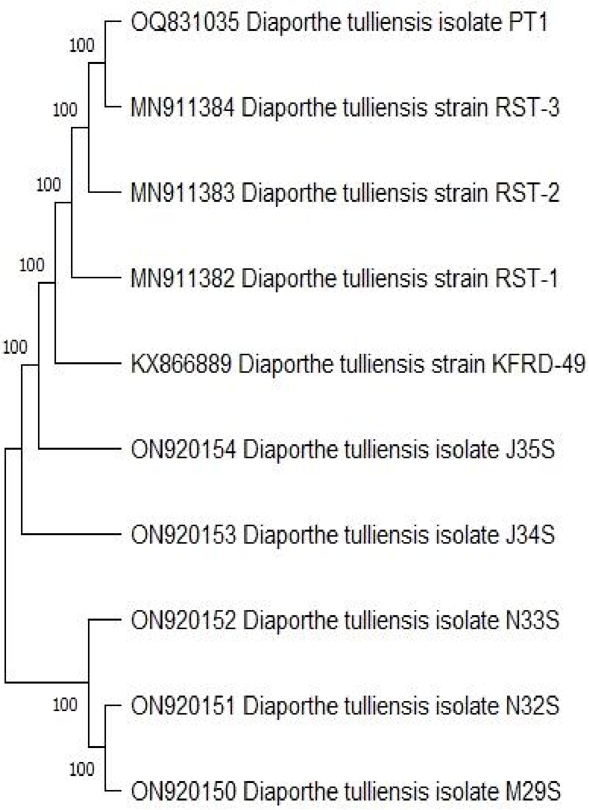

Fig 2 : Phylogenetic relationship among *Diaporthe tulliensis* strain using the Neighbor-Joining method. Relevant bootstrap values (expressed as percentage of 1000 replicates) are shown at branch point

morphological identification and BLASTn analyses.

To the best of our knowledge, this is the first report of *Diaporthe tulliensis* R.G. Shivas, Vawdrey & Y.P. Tan. as a fungal pathogen of patharchur leaves. *Diaporthe tulliensis* has been reported to cause leaf spots on coffee inChina (Gorg *et al.* 2020) and on *Parthenocissus tricuspidata* in Taiwan (Huang*et al.* 2021), and (Shabong and Kayang, 2023) on betel vine in India.

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DECLARATION

Conflict of Interest. Authors declare no conflict of interest.

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