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NEW DISEASE REPORT

First report of *Diaporthe tulliensis* causing leaf spot of betel vine

K. Shabong | **H. Kayang**

Microbial Ecology Laboratory, Department of Botany, North-Eastern Hill University, Shillong, Meghalaya, India

Correspondence
K. Shabong, Microbial Ecology Laboratory, Department of Botany, North-Eastern Hill University, Shillong-793022, Meghalaya, India.
Email khamti21@gmail.com

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The betel vine (*Piper betle*) is grown in protected areas under the humid and shaded conditions required for the plant's growth. This damp, shady environment favours the spread of numerous diseases, particularly leaf spot disease (Chattopadhayay & Maity, 1990). During 2019–2022, diseased betel leaves were collected from the East Khasi Hills and Ri Bhoi districts of Meghalaya, India. The leaves had spots that were irregular in shape and size, light to dark brown and surrounded by a chlorotic yellow halo, which gradually spread to the leaf centre followed by yellowing, drying and drooping of the whole leaf. An area of approximately of 8 m^2 was affected and 5–20% of the plants had symptoms, depending on the year. The number of leaf spots increased with temperature and humidity.

To identify the causal agent, lesions (5-10 mm in diameter) were cut from ten infected leaves (Figure 1), surface disinfected with 70% ethanol, rinsed three times in sterile distilled water and then plated on potato dextrose agar (PDA) amended with 30mg/ml of streptomycin sulphate. The same colony type was obtained from all leaves which was white producing yellowish-green pigments and the reverse olive green to brown after seven days (Figure 2A-B) and after 30 days of incubation at 30°C, produced semi-immersed black pycnidial conidiomata which exuded pale yellow to black conidial droplets (Figure 2C). The α-conidia were one to two-celled, hyaline, oval to fusiform, guttulate and 5.3×2.5 μm (Figure 2D), conidiophores hyaline but no β- and γ-conidia were observed (Li *et al*., 2022), and conidiogenous cells were hyaline and septate (Figure 2E-F). The identification of the fungus was confirmed by sequence analysis using ITS1 and ITS4 primers (White et al., 1990) (GenBank Accession No. OQ402579). BLAST analysis showed 100% identity with sequences of *Diaporthe tulliensis* (e.g. MN911384). In phylogenetic analysis, the isolate in the current study clustered with

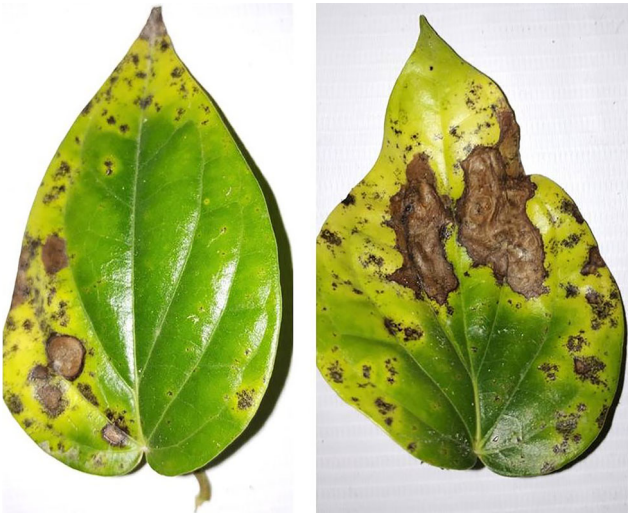


FIGURE 1 Leaf spot symptoms on infected betel leaves observed in the field in Meghalaya, India

D. tulliensis using the neighbour-joining method, showing 100% identity (Figure 3).

To confirm pathogenicity, the isolate was grown in potato dextrose broth at 28°C for seven days, then inoculated onto five healthy betel leaves using pin-prick inoculation with 10 μl spore suspension (1×10^5 spores/ml). The untreated control did not show any symptoms, however, the inoculated plants had brown necrotic spots followed by yellowing after four days (Figure 4). On PDA, cultures isolated from infected plant leaf lesions exhibited the same morphology as *D. tul-*

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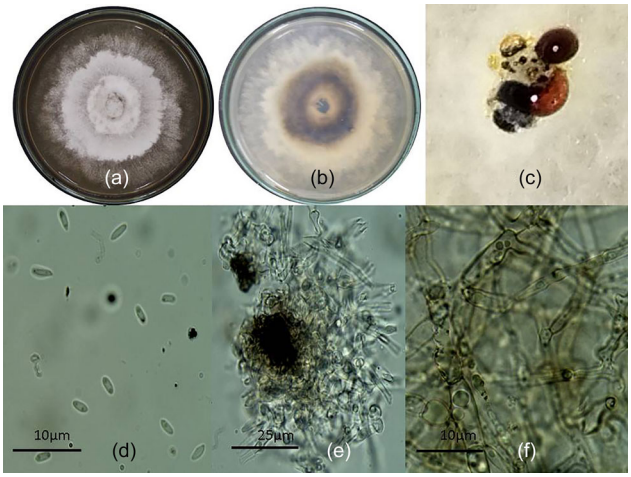


FIGURE 2 *Diaporthe tulliensis* growing on potato dextrose agar: (A) front and (B) reverse; and (C) pycnidia conidiomata, (D) alpha conidia and (E-F) conidiogenous cells

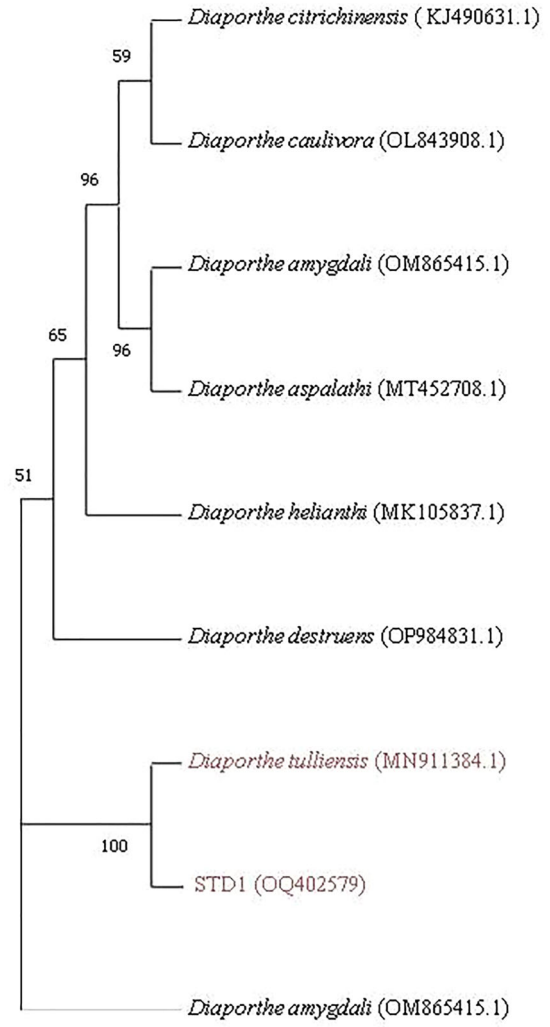


FIGURE 3 Phylogenetic relationships among *Diaporthe* spp, using the neighbour-joining method and Kimura 2-parameter model. Relevant bootstrap values (expressed as a percentage of 1000 replicates) are shown at branch points.



FIGURE 4 Symptoms caused by *Diaporthe tulliensis* on a healthy betel leaf four days after inoculation

liensis and similar conidia were discovered during microscopic analysis subsequently satisfying Koch's postulates.

To the best of our knowledge, this is the first report of *D. tulliensis* as a fungal pathogen of betel leaves. *Diaporthe tulliensis* has been reported to cause leaf spots on coffee in China (Gorg *et al*., 2020) and on *Parthenocissus tricuspidata* in Taiwan (Huang et al., 2021), cacao pod rot in Puerto Rico (Serrato-Diaz *et al*., 2022), and stem canker of kiwifruit in China (Bai *et al*., 2017) and of jasmine in Taiwan (Hsu et al., 2023).

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ORCID

K. Shabong https://orcid.org/0000-0002-3113-8649

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