



NEW DISEASE REPORT

First report of Pestalotioid species causing foliar diseases on mangrove trees in Terengganu, Peninsular Malaysia

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mangrove, Neopestalotiopsis, Pseudopestalotiopsis

In 2017, a survey of the mangroves at the Universiti Malaysia Terengganu revealed the presence of unfamiliar leaf spots and lesions (Sahibu et al., 2020). To identify the causal organism, almost 4,000 mangrove leaves were collected from five different species (*Avicennia rumphi-*

ana, *Bruguiera cylindrica*, *Ceriops zippeliana*, *Rhizophora apiculata* and *R. mucronata*) in the Setiu and the Kerteh Wetlands. Leaves were examined and sorted according to the symptoms, i.e. leaf spot or blight (Figures 1–2).

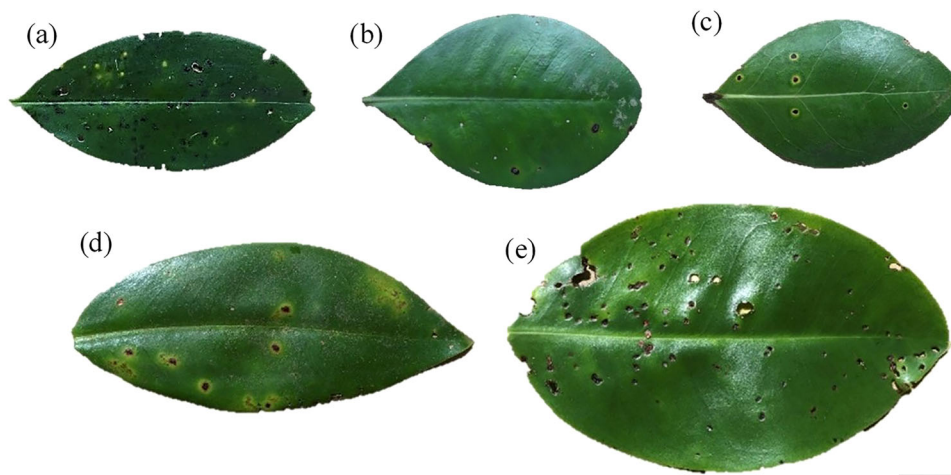


FIGURE 1 Leaf spot symptoms observed on different mangrove species. Embedded spots on (a) *Bruiguiera cylindrica* and (e) *Rhizophora mucronata*; sunken spots on (b) *Ceriops zippeliana*, (c) *Avicennia rumphiana* and (d) *Rhizophora apiculata*. Bar = 2 cm.

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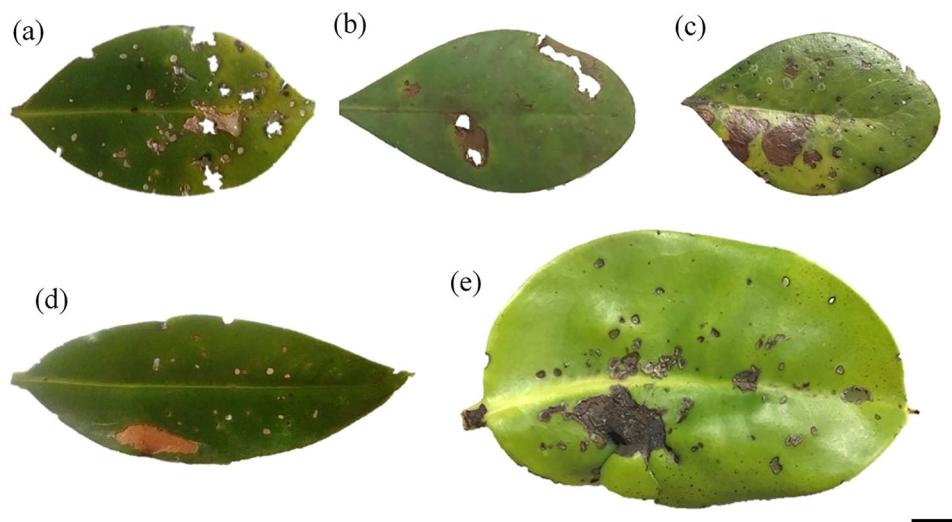


FIGURE 2 Leaf blight symptoms observed on different mangrove species. (a) *Bruiguiera cylindrica*, (b) *Ceriops zippeliana*, (c) *Avicennia rumphiana*, (d) *Rhizophora apiculata* and (e) *Rhizophora mucronata*. Bar = 1.9 cm.

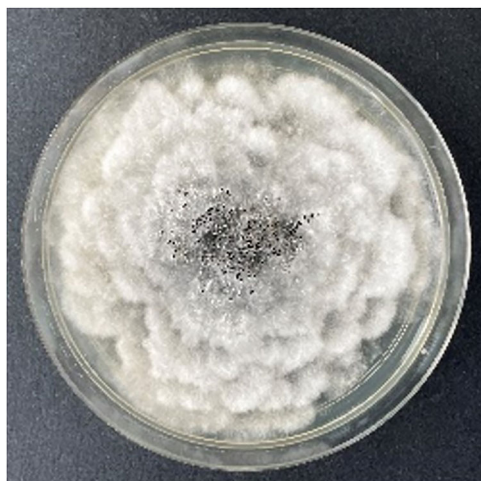


FIGURE 3 Fungal isolate on potato dextrose agar with creamy white mycelium and black fruiting bodies (pycnidia). Bar = 10 μ m.

Diseased leaves were cut into smaller pieces (1 cm diameter), surface sterilised (70% ethanol swab) and rinsed twice with sterilised distilled water for one minute, then put onto potato dextrose agar (PDA) and incubated for 10 days at 27°C. Pure cultures were obtained via single spore isolation. On PDA, creamy white mycelia with black pycnidia were formed (Figure 3). Conidia were ellipsoid, straight to slightly curved, separating into five-cells with a concolorous median cell (Figure 4a). Two knobbed apical appendages (Figure 4b), resembling the description of *Pestalotiopsis* spp. (Maharachchikumbura et al., 2014), were observed.

The genomic DNA of ten isolates was extracted using an Invisorb Spin Plant Mini Kit (STRATEC Molecular, Germany) and the internal transcribed spacer (ITS) region was amplified using the universal ITS1/ITS4 primers and sequenced (White et al., 1990). To confirm the species identity, two additional genes, Beta-tubulin (TUB) and the Translation Elongation Factor 1- α (TEF) were amplified and sequenced with the Bt2a/Bt2b and EF1-728F/EF2 primer pairs respectively. The sequences of all isolates had 99% identity with *Neopestalotiopsis saprophytica* (GenBank Accession Nos. OQ940399-OQ940402), *Neopestalotiopsis clavispora* (OQ940405, OQ940406 and OQ940474), *Neopestalotiopsis thailandica* (OQ940404) and *Pseudopestalotiopsis theae* (OQ940473). Reconstruction of the maximum likelihood phylogenetic tree was based on the combined of ITS, TUB and TEF sequences and reference isolates from GenBank (Figure 5).

Pathogenicity tests were conducted using a detached leaf assay (Omar et al., 2018). Healthy leaves were wounded using a fine needle and inoculated with a Pestalotioid-infested agar plug (5 mm diameter), whereas control leaves were treated with a sterile PDA plug. Three replicates were used for each isolate including the control. Leaves were kept in the dark in a sterile damp container for ten days at 25–27°C. The inoculated leaves developed lesions and blight symptoms resembling those in the field. No symptoms developed on control leaves. Koch's postulates were fulfilled by reisolating and identifying the pathogens.

Pestalotiopsis spp. were isolated previously as endophytic fungi on *Rhizophora* mangrove in India (Suryanarayanan et al., 1998) and were reported from *Rhizophora mangle* showing dieback symptoms in The Bahamas (Rossi, 2018). This is the first report of *Neopestalotiopsis*

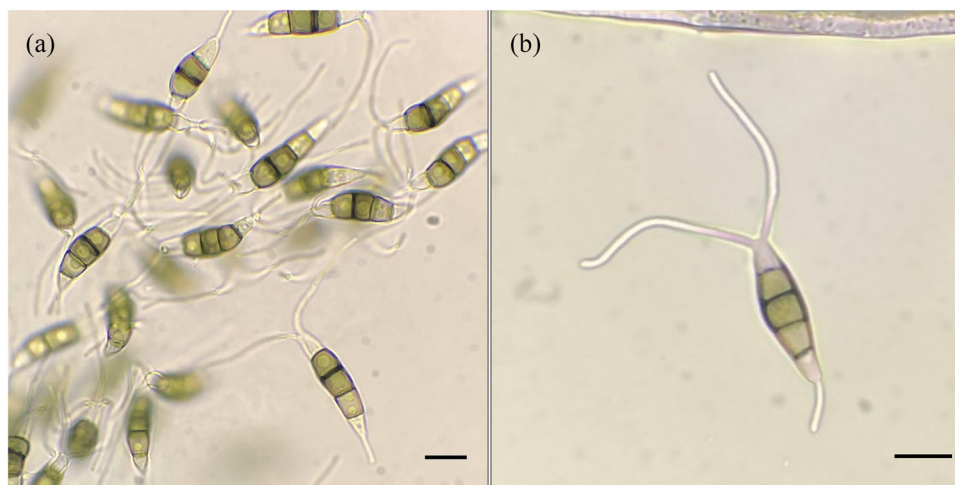


FIGURE 4 (a) Five-celled conidia with concolorous median cells and (b) two knobbed apical appendages. Bar = 10 µm.

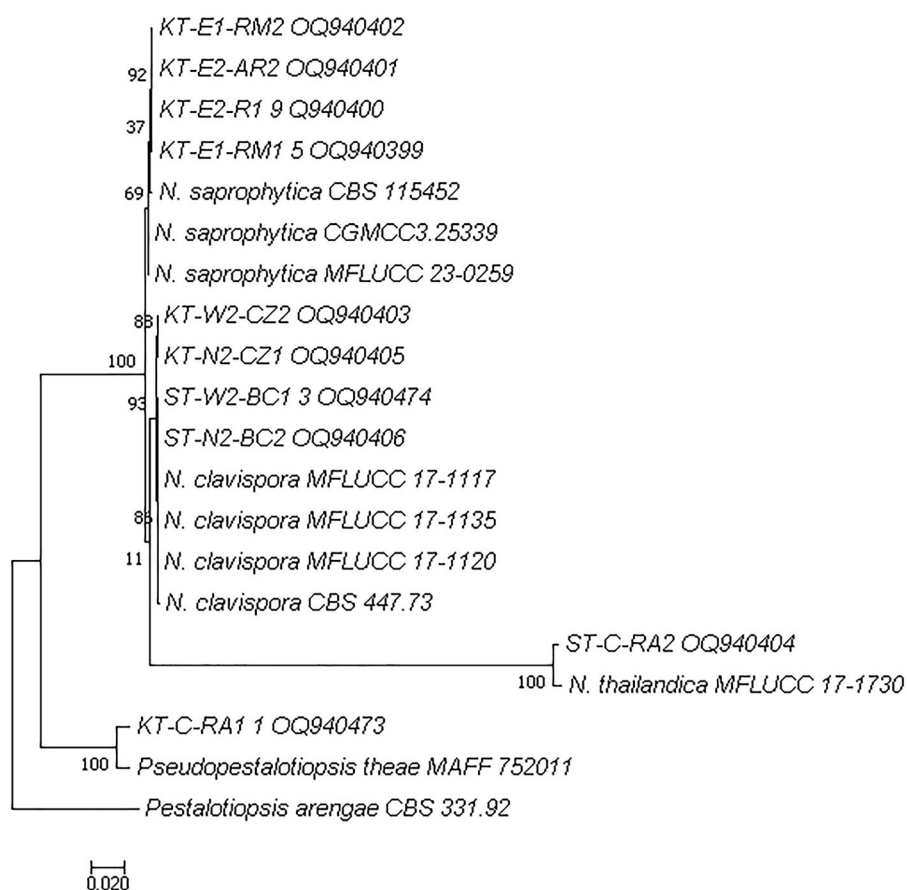


FIGURE 5 Maximum-likelihood tree based on the Tamura 3-parameter model of ten isolates based on the individual ITS, TUB and TEF sequences. The analysis involved 15 sequences. The numbers in the bootstrap test (1,000 replicates) are shown above the branches. *Pestalotiopsis arengae* strain CBS 331.92 was used as the outgroup. Scale bars represent the estimated number of substitutions per site.



spp. and *Pseudopestalotiopsis theae* causing disease symptoms on mangroves in Malaysia and this information will aid the country's disease monitoring programme.

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