Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Paramyothecium roridum* Causing Leaf Blight on Water Hyacinth (*Eichhornia crassipes*) in Malaysia

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Funding: This study was funded by Putra Grant from the University Putra Malaysia (UPM) (9675300). Plant Dis. 106:1296, 2022; published online as <https://doi.org/10.1094/PDIS-04-21-0704-PDN>. Accepted for publication 2 October 2021.

Water hyacinth (*Eichhornia crassipes*) is a free-floating aquatic plant and is also widely cultivated as an aquatic ornamental plant in Malaysia. In June 2018, a severe foliar disease with typical leaf blight symptoms were observed on leaves of water hyacinth plants (approximately 50%) in waterways adjacent to two rice fields located at Tanjung Karang and Sungai Besar, Selangor Province, Malaysia. Symptoms appeared as irregular necrotic lesions with concentric rings; later, lesions expanded to entire leaves and became blighted. Twenty symptomatic leaves were collected from two sampling locations. Symptomatic leaf tissue was cut into small pieces (5 by 5 mm), surface sterilized with 0.5% sodium hypochlorite (NaOCl) for 2 min, rinsed three times with sterile distilled water, plated on potato dextrose agar (PDA), and incubated at 25°C with a 12-h light-dark cycle for 7 days. Twenty single-spore isolates were recovered from sampled leaves. All isolates exhibited *Paramyothecium*-like morphology and two representative isolates, PR1 and PR2, were used for further studies. Fungal colonies were initially white aerial mycelia, with sporodochia bearing olivaceous green conidial masses forming on PDA after 5 days of incubation. Conidiogenous cells were phialidic, hyaline, smooth, straight to slightly curved, 13 to 20 by 1.0 to 1.8 µm, and setae were absent. Conidia were aseptate, hyaline to pale green, smooth, cylindrical to ellipsoidal with rounded ends, and measured 5.8 to 8.0 µm by 1.8 to 2.2 µm ($n = 50$). These morphological characteristics were consistent with the description of *Paramyothecium roridum* (Tode) L. Lombard & Crous (Lombard et al. 2016). Total genomic DNA of the isolates was extracted from fresh mycelium using a DNeasy Plant Mini kit (Qiagen). The internal transcribed spacer (ITS) and calmodulin (*cmdA*) gene regions were amplified using the ITS5/ITS4 (White et al. 1990) and CAL-228F/CAL2Rd primer sets, respectively (Carbone and Kohn 1999; Groenewald et al. 2013). BLASTn

analysis showed that the ITS and *cmdA* sequences of the isolates had 100% identity with *P. roridum* ex-epitype strain CBS 357.89 (GenBank accession nos. KU846300 and KU846270, respectively). The resulting sequences were deposited in GenBank (ITS accession nos. MW850370 and MW850371; *cmdA* accession nos. MW854363 and MW854364). Pathogenicity tests of the two isolates were performed by spray inoculation on healthy leaves of each five potted water hyacinth plants using a 3-ml conidial suspension (1×10^6 conidia/ml) produced on 7-day-old PDA cultures incubated at 25°C with a 12-h light/dark cycle. Five potted water hyacinth plants inoculated with sterile water served as controls. Inoculated plants were covered with plastic bags for 48 h to maintain high humidity and kept in a growth chamber for 2 weeks at $25 \pm 1^\circ\text{C}$, 95% relative humidity, and a 12-h light/dark period. The experiment was repeated twice. Eight days postinoculation, inoculated leaves developed symptoms (necrotic brown lesions) similar to those observed in the field, while control leaves remained asymptomatic. After 2 weeks of inoculation, lesions enlarged into severe blighting until all leaves died. *P. roridum* was reisolated from randomly selected symptomatic tissues and verified by morphology and sequencing of ITS (MZ675387 and MZ706462) and *cmdA* (MZ686706 and MZ712041) loci, confirming Koch's postulates. The fungus was not reisolated from noninoculated control plants. *P. roridum* is distributed on a wide range of plants (Farr and Rossman 2021) and has been reported to cause leaf spot of water hyacinth in Nigeria (Okunowo et al. 2013) and Sri Lanka (Adikaram and Yakandawala 2020). To our knowledge, this is the first report of *P. roridum* causing leaf blight of water hyacinth in Malaysia. This disease is an emerging threat to water hyacinth, and it reduces leaf quality; therefore, appropriate management should be developed to control this disease.

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The author(s) declare no conflict of interest.

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Keywords: *Paramyothecium roridum*, *Eichhornia crassipes*, water hyacinth, leaf blight

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