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First Report of *Pseudopestalotiopsis ampullacea* Causing Leaf Spot on *Hevea brasiliensis* in Malaysia

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Rubber is an economically important crop cultivated extensively in Malaysia for latex production. In March 2024, necrotic spots were observed on mature leaves of rubber plants (clone RRIM2004) during surveys in two rubber plantations, in Sungai Buloh, Selangor state and Kota Tinggi, Johor state, with a disease incidence of 80%. Initial symptoms on leaves appeared as light-yellow, circular, semi-circular to irregular lesions (2–5 mm in diameter) on the adaxial leaf surface, which gradually changed to brown and grey-white spots (Figure 1). Diseased leaves became blighted and the plants defoliated as the disease progressed.

To identify the pathogen, fragments (5 × 5 mm) were excised from the margin of the diseased leaf tissues, surface-sterilised with 1% sodium hypochlorite solution for three minutes, rinsed three times with sterile distilled water, placed on potato dextrose agar (PDA) and incubated at 28°C with a 12 h photoperiod for 7 days. Ten single-spore isolates were obtained from sampled leaves, all isolates exhibited a *Pseudopestalotiopsis*-like morphology and two representative isolates (PA1 and PA2) were selected for further study. Colonies on PDA were whitish with dense aerial mycelia, forming black gregarious conidiomata and the reverse side was whitish to pale yellow (Figure 2). Conidia were fusoid to ellipsoid, straight to slightly curved, 4-septate, ranging from 21 to 30 ±6.5–9 µm ($n = 30$) and septa darker than the rest of the cell (Figure 3). The basal cells were conic with a truncate base, hyaline and thin-walled, 2.5–5.0 µm long. Three median cells were doliiform, 13.5 to 19.5 µm long, hyaline, subcylindrical, thin-walled, with 2–3 tubular apical appendages arising from the apical crest, unbranched, filiform, 17–25 µm long. The basal appendages were singular, tubular, unbranched, centric, 3.5–7.0 µm long. On the

FIGURE 1 | Leaf spot symptom on naturally infected rubber leaves (*Hevea brasiliensis*) associated with *Pseudopestalotiopsis ampullacea*.

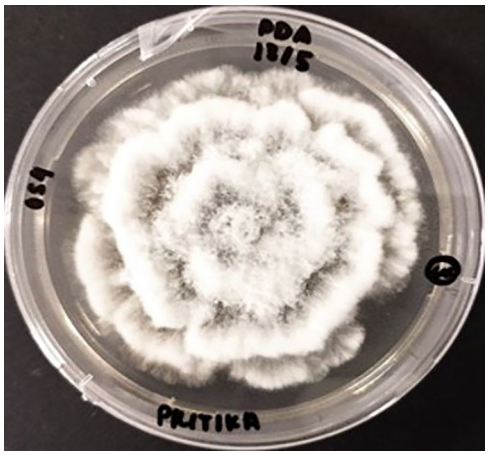

FIGURE 2 | Mycelial growth of *Pseudopestalotiopsis ampullacea* on potato dextrose agar media after 5 days.

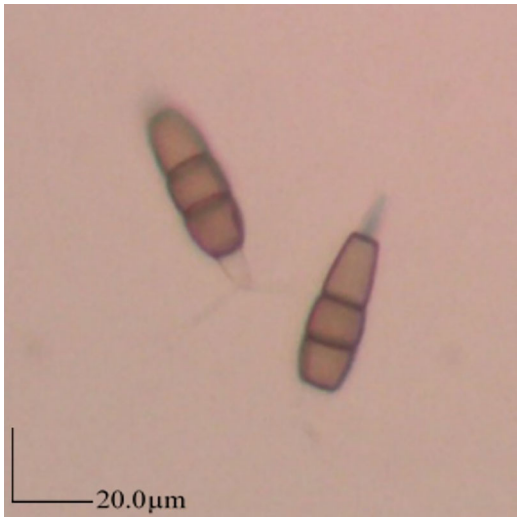

FIGURE 3 | Four-septate conidia of *Pseudopestalotiopsis ampullacea*. Bar = 20 µm.

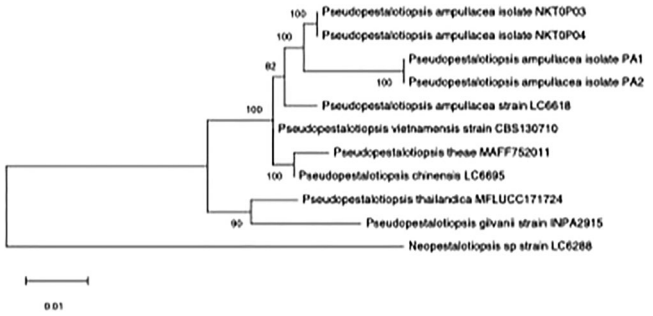

FIGURE 4 | Maximum likelihood phylogeny generated from concatenated sequences of two loci, ITS-TEF1-α sequences. Evolutionary analyses were conducted in MEGA12. Node values represent support based on 1000 bootstrap replicates. Scale bar denotes the number of substitutions per site. *Neopestalotiopsis* sp. LC6288 was used as an outgroup.

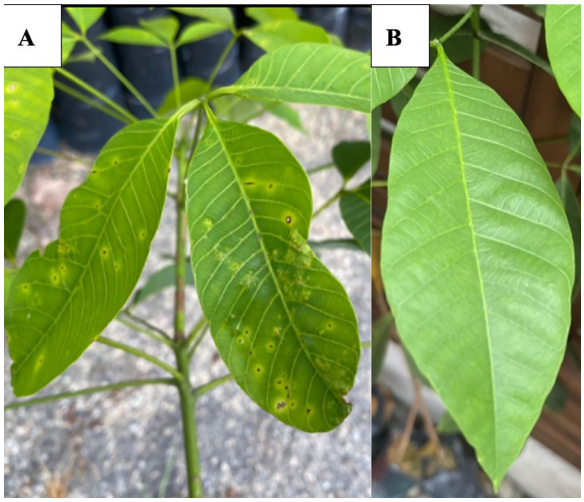

FIGURE 5 | Leaves of rubber seedlings (clone RRIM2004) seven days after (A) inoculation with isolate PA1 and (B) mock inoculation with sterile water (control treatment).

basis of morphology, both representative isolates were identified as *Pseudopestalotiopsis* (Maharachchikumbura et al. 2014).

The internal transcribed spacer (ITS) region of rDNA and translation elongation factor 1-alpha (TEF1-α) gene of isolates PA1 and PA2 were amplified using the ITS5/ITS4 and EF1-728F/EF1-986R primer set, respectively (White et al. 1990; Carbone and Kohn 1999). BLASTn analysis of the resulting ITS and TEF1-α sequences indicated 99% identity to ex-holotype *Pseudopestalotiopsis ampullacea* strain LC6618. The ITS (GenBank Accession Nos. PP779714 and PP779715) and TEF1-α (PP785033 and PP785034) sequences were deposited in the GenBank databases. Phylogenetic analysis using the maximum likelihood analysis based on the concatenated ITS-TEF1-α indicated that the *Ps. ampullacea* PA1 and PA2 isolates form a strongly supported clade (82 bootstrap value) to the ex-holotype culture of *Ps. ampullacea* LC6618 and both isolates were most closely related to other *Pseudopestalotiopsis* species (Figure 4) (Kumar et al. 2024).

Five healthy leaves from 6-month-old rubber plants (clone RRIM2004) were inoculated with either isolate PA1 or PA2 according to Liu et al. (2025). Control leaves were mock-inoculated using sterile water. Seven days post-inoculation, necrotic lesions developed on inoculated leaves, closely resembling symptoms observed on naturally infected rubber leaves in the field, whereas the control leaves remained asymptomatic (Figure 5). *Pseudopestalotiopsis ampullacea* was re-isolated from all symptomatic tissues, verified by molecular identification, confirming Koch's postulates.

This is the first report of *Ps. ampullacea* causing leaf spot symptoms on *Hevea brasiliensis* in Malaysia. The pathogen is primarily known to infect palm species, particularly oil palm (Ismail et al. 2017). The occurrence of this disease needs to be monitored because it poses a significant threat with the potential to reduce latex production by 28%–46% (Kusdiana and Saputra 2022), adversely affecting overall yield and profitability. Therefore, preventive strategies need to be developed to reduce the incidence of the disease in the field.

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