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First Report of *Trichoderma longibrachiatum* on *Phoenix dactylifera* in Pakistan

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FIGURE 1 | (a) Symptoms of *Trichoderma longibrachiatum* on leaves of *Phoenix dactylifera*; and (b) infection observed under a stereomicroscope.

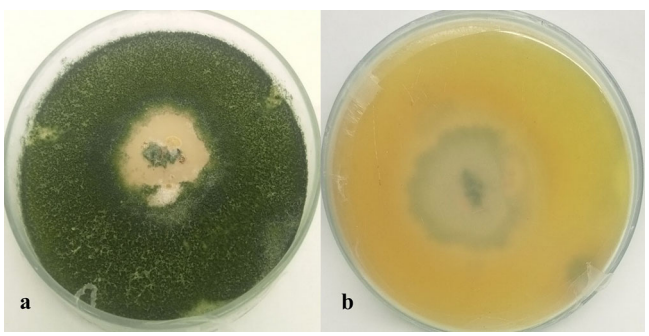


FIGURE 2 | Colony of *Trichoderma longibrachiatum* on potato dextrose agar pictured from the (a) top and (b) bottom.

The date palm (*Phoenix dactylifera*) is mainly grown in arid and semi-arid regions of the world, including countries in South Asia, America and North Africa. Pakistan ranks sixth among date-producing countries (Majid et al. 2023). In the rainy season of 2023, *P. dactylifera* trees showing symptoms of small black spots and circular lesions on leaves were observed in Punjab, Pakistan. When the trees were cut down, the leaves had black spots (Figure 1). Segments of 1.5 cm were taken from the border zone between healthy and discoloured branches.

Branches were surface sterilised with 70% alcohol and 1.5% sodium hypochlorite, rinsed with sterile water, and small sections of branch taken from the leading edge of lesions and plated onto potato dextrose agar (PDA). The resulting fungi were transferred to new PDA plates and incubated at 25°C ± 2°C for 21 days. Colonies growing on PDA had a woolly or cottony texture and diffuse yellow pigment. The colonies were off-white or white coloured initially, which changed to a greyish-green hue after 8 days (Figure 2). Conidiophores were typically branched, branching at wide angles. Phialides, flask-shaped structures that produce conidia, were positioned at wide angles to the conidiophores, 5.0–10.0 × 2.0–3.0 μm. Conidia were typically clustered at the tips of the phialides and were yellow-green or white in colour and 2.74–4.17 × 5.58–6.85 μm in diameter (Figure 3). The size range and shape of these conidia are similar to the description given by Sarsaiya et al. (2019).

Primer pairs ITS1/ ITS4 were used to amplify ITS1, 5.8S, ITS2 regions of this isolate and BLASTn analysis showed 96% identity with ITS reference sequences of *Trichoderma*

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FIGURE 3 | (a) Conidiophores with phialides (bar = 10 μm) and (b) conidia (bar = 4 μm).

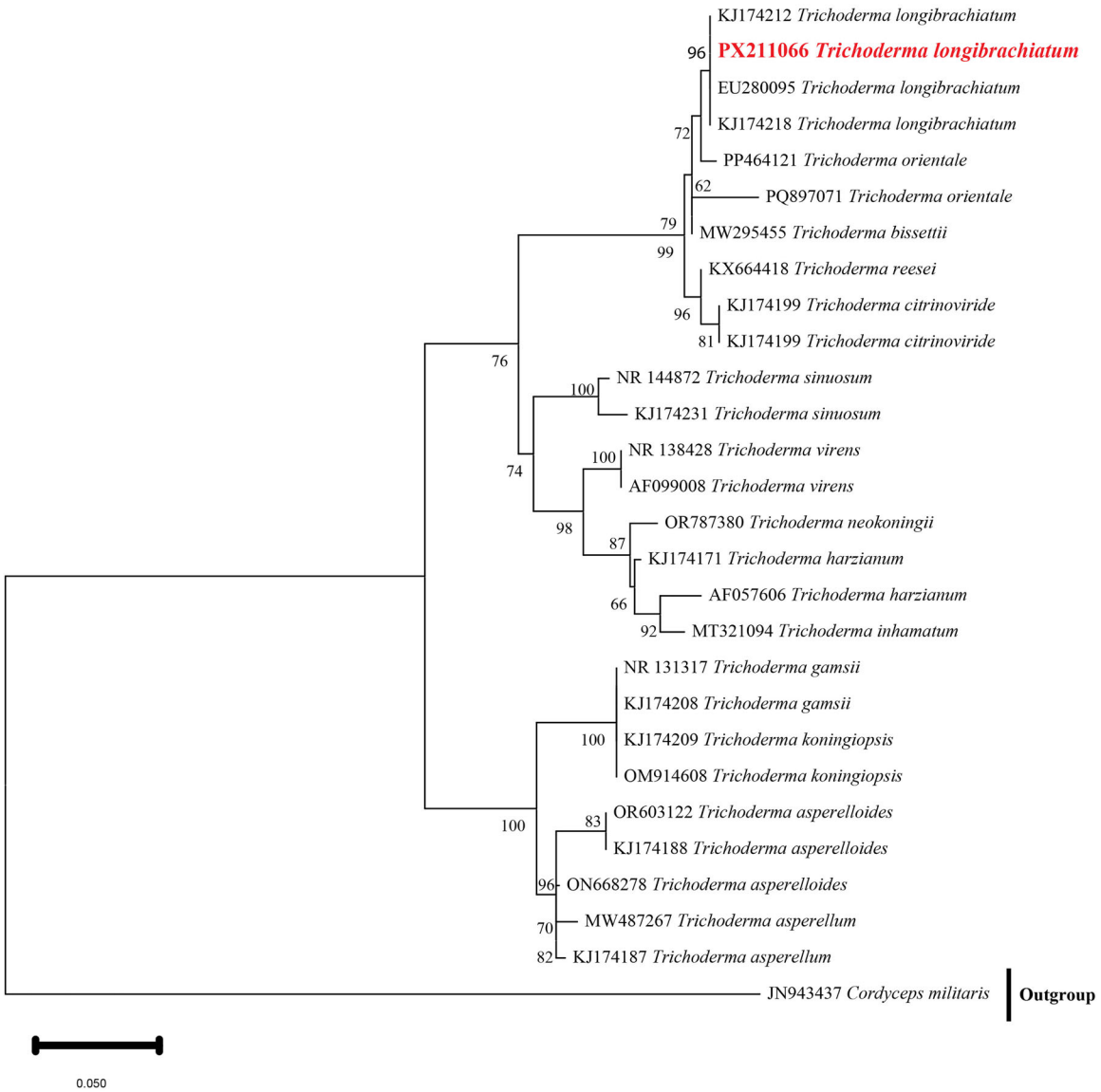


FIGURE 4 | Maximum likelihood tree based on ITS DNA sequences of representative isolates of *Trichoderma longibrachiatum*. Inference of phylogeny was conducted in MEGA 11 (Tamura et al. 2021) using the General Time Reversible model. The isolate from this study is highlighted. *Cordyceps militaris* was used as an outgroup. Bootstrap values (1000 replications) are indicated on the nodes. Scale bar indicates number of substitutions per site.

longibrachiatum (GenBank Accession Nos. KJ174212, Eu280095 and KJ174218). Based on the morphological and molecular analyses, the isolate was identified as *T. longibrachiatum* and sequences were deposited in GenBank (PX211066). A phylogenetic tree based on neighbour-joining analysis of sequences derived from the ITS region was produced, based on 1000 bootstrap replicates of *T. longibrachiatum* and other *Trichoderma* sequences (Figure 4).

To test the pathogenicity of the isolate, disease-free branches were inoculated with the isolate of *T. longibrachiatum*. Date palms were wounded with a sterilised blade near the roots and mycelia of the isolate were placed into the wound with a sterilised brush and sealed with plastic film, while the control received sterile water. Branches were maintained at 22°C and 12 h day/light in a greenhouse. Ninety days post-inoculation, branches with small black spots and circular lesion symptoms were recorded, whereas the control remained symptom-free. *T. longibrachiatum* was re-isolated from infected branches and identified morphologically and molecularly as previously described, fulfilling Koch's postulates.

In China, *T. longibrachiatum* has been reported to cause black circular spots on leaves of *Dendrobium nobile* and green mould disease on *Ganoderma lingzhi* (Sarsaiya et al. 2019; Zhang et al. 2019). The small black spots and circular lesions on *P. dactylifera* caused by *T. longibrachiatum* have not been previously reported. To the best of our knowledge, this is the first report of *T. longibrachiatum* as a plant pathogen on *P. dactylifera* in Pakistan and globally.

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