

First report of *Trichoderma lixii* causing leaf spot of *Ceropegia vincaefolia* in India

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Lantern flower (*Ceropegia vincaefolia*, Apocynaceae) is a perennial herbaceous climber which is endemic to the Western Ghats mountain range, especially the Rajasthan, Maharashtra and Kerala states of India. The tubers of *C. vincaefolia* have nutritional value due to their starch, gum, sugar, albuminoid, fat, crude fibre and mineral content, and the leaves are used as a leafy vegetable (Chavan *et al.*, 2018). The flow-



FIGURE 1 Leaf spot caused by *Trichoderma lixii* on leaves of *Ceropegia vincaefolia*

ers have horticultural value due to their unique phenotype and diverse colouring of the flowers (Chavan *et al.*, 2018).

During September 2020 and September 2021, irregular, white-greyish spots (10 to 15 mm) were observed on the adaxial (but not abaxial) surface of the leaves of several *C. vincaefolia* plants growing naturally on the Kaas Plateau (17.721794° N and 73.810873° E) near the city of Satara, Maharashtra, India (Fig. 1). The infection led to chlorosis and subsequent defoliation. Infected leaves were collected and a fungus was isolated in pure culture on potato dextrose agar medium by the fungal tip method. The fungus displayed a characteristic slow early growth of white cottony mycelium, rapidly turning into green and greyish-green colonies, while the bottom side of the plates remained colourless or light yellow (Fig. 2). After 15 days' incubation, grey-greenish and globose to sub-globose conidia (3.1–3.6 × 2.5–3.1 µm) appeared on pyramidal conidiophores (Fig. 3). The 5.8S rRNA of five fungal isolates was amplified, sequenced and submitted to GenBank (Accession No. ON530942.1). BLASTn analysis showed 100% identity with sequences of *Trichoderma lixii* (NR131264.1) (Fig. 4). Based on the morphological and molecular data the fungus was identified as *T. lixii*.

Koch's postulates were fulfilled by spray inoculating a suspension of conidia mixed from the five isolates (approx. 8,000 conidia/ml) on the adaxial and abaxial surface of the leaves of twenty plants of *C. vincaefolia*. The leaves of five healthy plants were sprayed with sterile water to serve as controls. After 12 days in a greenhouse (10–18°C), the inoculated leaves showed the symptoms described above on the adaxial surface only, as observed in the field. The tests were repeated three times in the greenhouse and results confirmed the pathogenicity of *T. lixii* on *C. vincaefolia*. The pathogen was re-isolated from the inoculated leaves and identified morphologically.

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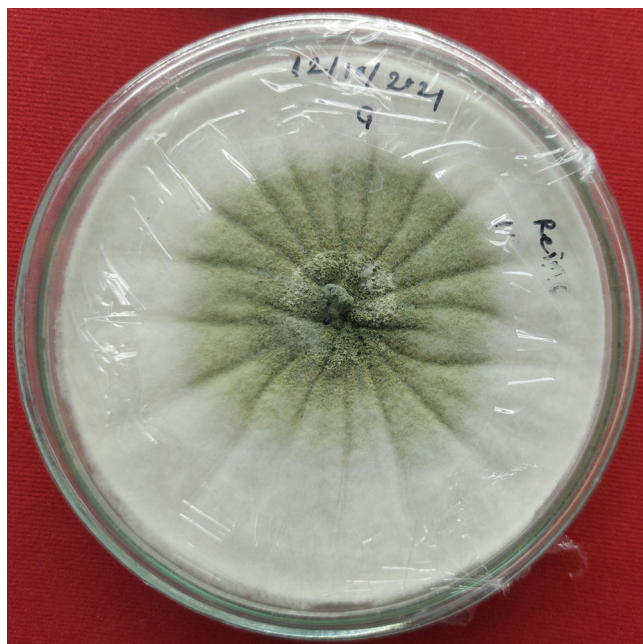


FIGURE 2 Colony characteristics of *Trichoderma lixii* on potato dextrose agar medium

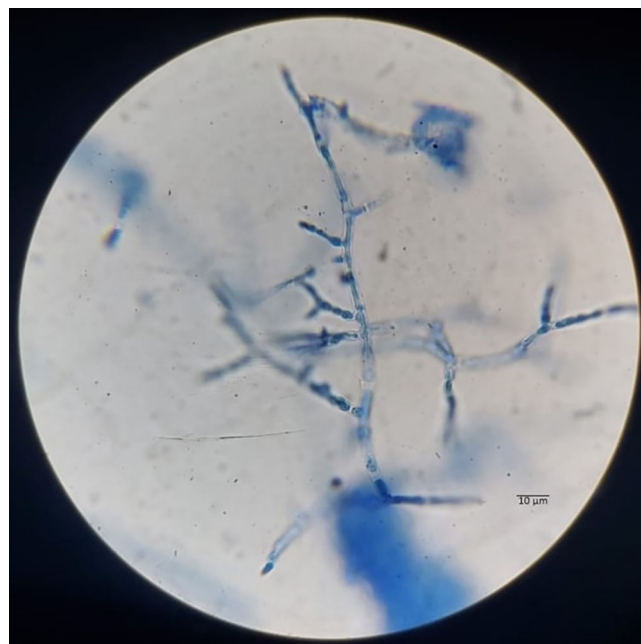


FIGURE 3 Microscopic features of *Trichoderma lixii*

Recent studies have reported that *Trichoderma* spp. cause disease in a number of plant species, for example, *T. afroharzianum* causes seed rot on maize in Italy (Sanna et al., 2022). Though *T. lixii* causes leaf spot of *C. vincaefolia*, it has also been identified as an endophyte of *Bacopa*

monnieri (Katoch et al., 2019) and it supports the vigorous and healthy growth of plants (tomatoes, strawberries and vines) in Algeria (Ham-mad et al., 2021). To our knowledge this is the first report confirming *T. lixii* causing leaf spot disease of *C. vincaefolia* in India.

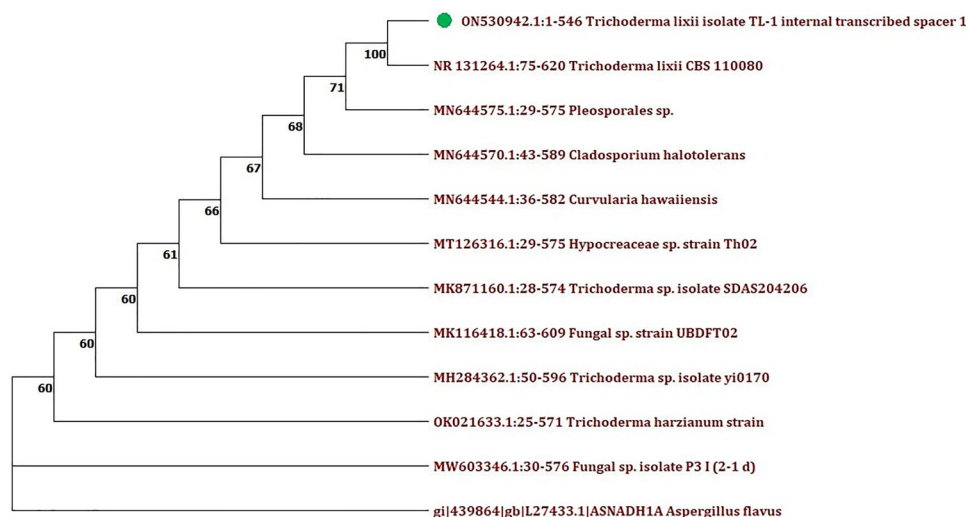


FIGURE 4 Evolutionary relationship of *T. lixii* based on the ITS (5.8s) region sequences. The evolutionary history was inferred using the neighbour-joining method. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches. The evolutionary distances were computed using the Kimura 2-parameter method and are in the units of the number of base substitutions per site. There were a total of 995 positions in the final dataset. Evolutionary analyses were conducted in MEGA11

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REFERENCES

- Chavan, J.J., Gaikwad, N.B., Dixit, G.B., Yadav, S.R. & Bapat, V.A. (2018) Biotechnological interventions for propagation, conservation and improvement of 'Lantern Flowers' (*Ceropegia* spp.). *South African Journal of Botany*, 114, 192–216. <https://doi.org/10.1016/j.sajb.2017.10.021>
- Hammad, M., Guillemette, T., Alem, M., Bastide, F. & Louanchi, M. (2021) First report of three species of *Trichoderma* isolated from the rhizosphere in Algeria and the high antagonistic effect of *Trichoderma brevicompactum* to control grey mould disease of tomato. *Egyptian Journal of Biological Pest Control*, 31, 85. <https://doi.org/10.1186/s41938-021-00423-4>
- Katoch, M., Singh, D., Kapoor, K.K. & Vishwakarma, R.A. (2019) *Trichoderma lixii* (IIIM-B4), an endophyte of *Bacopa monnieri* L. producing peptaibols. *BMC Microbiology*, 19, 98.
- Sanna, M., Pugliese, M., Gullino, M.L. & Mezzalama, M. (2022) First report of *Trichoderma afroharzianum* causing seed rot on maize in Italy. *Plant Disease*, 106, 1982. <https://doi.org/10.1094/PDIS-12-21-2697-PDN>

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