First report of *Curvularia lunata* leaf spot on *Trifolium alexandrinum* in Pakistan

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Trifolium alexandrinum (Berseem clover) is major winter fodder crop of Pakistan. Symptomatic leaves were collected from farmer field (31.1704° N, 72.7097° E) with 47% disease incidence during 2017, V-shaped brownish spots ranging 0.8 to 2.0 cm in diameter on leaflets, which later merged and had a black or burning appearance on severely infected leaves. Symptomatic leaves were cut, surface disinfected, placed onto potato dextrose agar (PDA) culture medium and incubated at 26°C. Fungal cultures were purified using a single hyphal tip technique. A seven day culture was grayish black in color and the reverse side was black in the center and lighter towards margin with branched, septate and smooth walled brownish hyphae. Conidiophores were unbranched with septations and dark brown in color. Conidia were dark brown in color, boat/hocked shaped with septations, with rounded tips and 26–33 × 12–15 µm in size (Ellis 1971; Manamgoda et al. 2015). This isolate was identified as *Curvularia lunata* based on morphological characteristics. Three loci, i.e. internal transcribed spacer region (ITS), RNA polymerase II second largest subunit (RPB2) and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) were sequenced and showed 100% similarity with strain CBS 730.96. The sequences were deposited in GenBank with accession numbers MN971670, MN972619 and MN972621 respectively. The characterized culture was deposited in the Fungal Molecular Biology Culture Collection (FMB-CCUAF) as FMB0201. Koch's postulates were completed thrice for pathogenicity confirmation and conducted as follows. Conidial suspension (1×10^6 spores/mL) of *C. lunata*

from seven-day-old cultures was applied to mechanically injured leaflets of Berseem plants. Control plants were treated with sterile water, and all plants were enclosed with polythene bags for one day. Lesions similar to those observed in the field appeared on inoculated leaves two weeks post inoculation. Control plants were asymptomatic. Based on morphological and molecular characterization the pathogen was identified as *C. lunata*. To our knowledge, this is the first report of *C. lunata* causing leaf spot of Berseem clover in Pakistan.

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Compliance with ethical standards

Conflict of interest All authors declare that they have no conflict of interests.

Ethical statement This article does not contain any studies with human participants or animals.

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