

First report of sunflower leaf spot caused by *Curvularia lunata* under temperate conditions of Kashmir, India

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In a disease survey of sunflower (*Helianthus annuus* L.) fields of Kashmir Province, India during April 2022, leaf spot disease was found on the crop with an average disease severity of 26%. Symptoms of the disease first appeared on leaves as small reddish-brown spots less than 1mm which increased in size up to 10 mm in diameter, turned brown with yellow halo and round. The spots enlarged and coalesced and developed large necrotic areas resulting in leaf blight. Severe infection caused yellowing and desiccation of whole leaf (Fig. 1). The disease also developed on stems as eye shaped light brown to black lesions that coalesced to form large areas of deceased tissues. The causal pathogen was isolated from collected symptomatic leaves and then purified by single spore isolation method (Johnston and Booth, 1983).



Fig.1: Disease symptoms of sunflower leaf spot under field conditions

The pathogenicity of the isolated fungus was confirmed by proving Koch's postulates on detached leaves (Plate-a). Spore suspension (4×10^6 spores ml^{-1}) prepared from 15 days old culture was used for inoculation. The fungus in pure culture on potato dextrose agar medium developed colonies which were olive brown to black with velvety texture and smooth margins (Plate-b). The fungus developed olive brown, branched and septate mycelium and the hyphae was hyaline about 2-4 μm in width. Conidia developed on unbranched septate

conidiophores. (Plate-d). The conidia were septate with 2-3 transverse septa, with large and curved central cells, smooth walled, black to brown in colour, straight to pyriform in shape and $21-30 \times 8.5-12.0 \mu\text{m}$ in size (Plate-c).

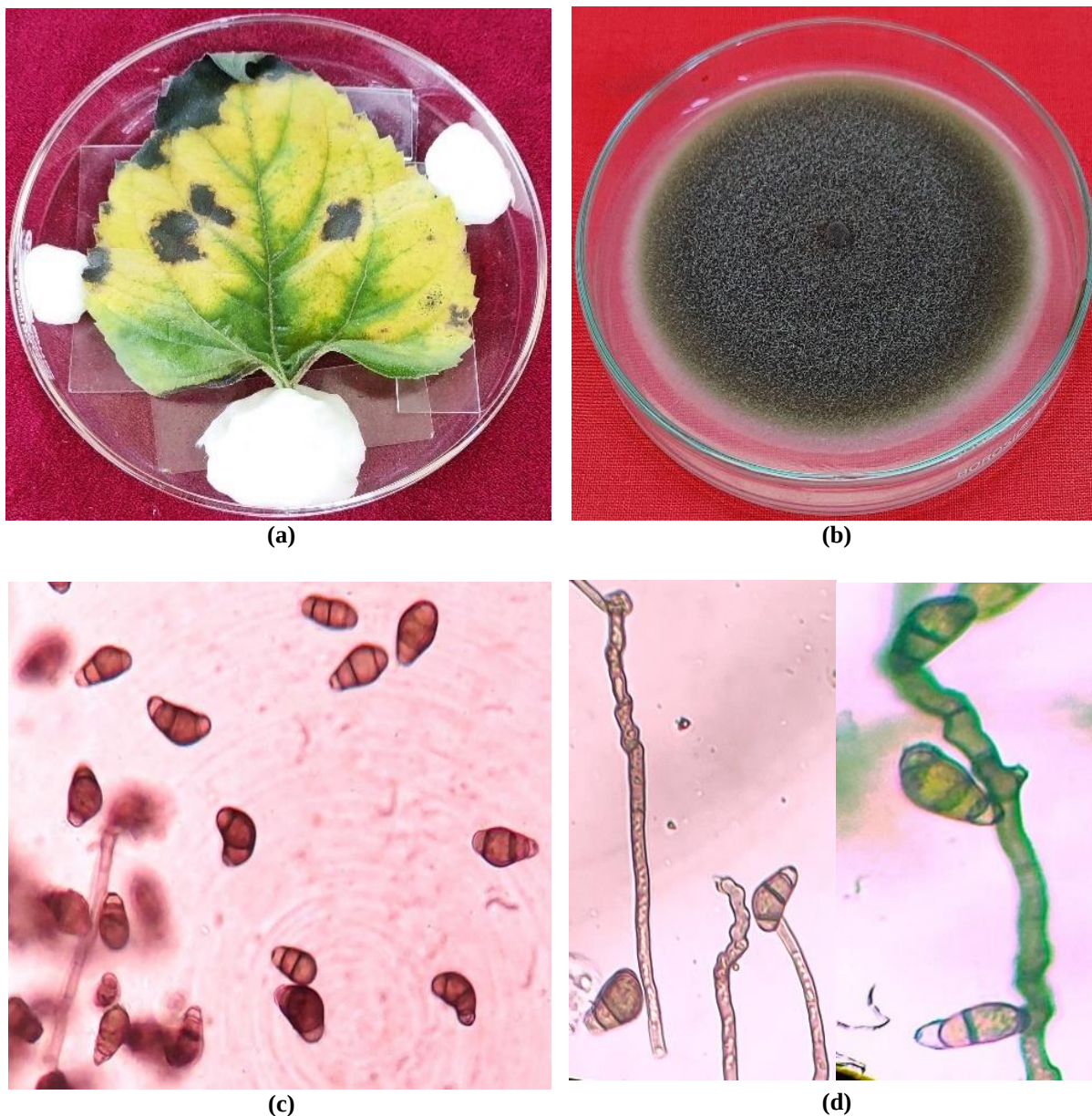


Fig. 2: Morphocultural characteristics of *Curvularia lunata* (a) Pathogenicity test on detached leaf, (b) 10 days old culture plate of *Curvularia lunata* on PDA, (c) Conidia, (d) Conidial development on unbranched conidiophore

Conidiophores were geniculate at the apical region, septate, black to dark brown in colour and $19-132 \times 1.5-5 \mu\text{m}$ in size. (Plate-d). The DNA of isolated pathogen was extracted from fungal mycelium. The internal transcribed spacer region of the DNA was amplified using ITS1 and ITS4 primers (White et al., 1990) and sequenced. The sequenced amplicons were queried in NCBI-BLAST with 99.64% and 98.38% similarity to *Curvularia lunata* (Wakker) Boedjin (Macri and Lenna, 1974) with ITS1 and ITS4 respectively (Fig.3). The DNA sequence of identified pathogen was deposited in GenBank corresponding with the accession numbers ON668436 and ON668437. Based on colony and morphological characters besides the pathogenicity and confirmation at molecular level, the isolated fungus was identified as *Curvularia lunata* (Wakker) Boedjin (Ellis, 1971) and is apparently the first report of leaf spot of sunflower under temperate conditions of Kashmir, India.

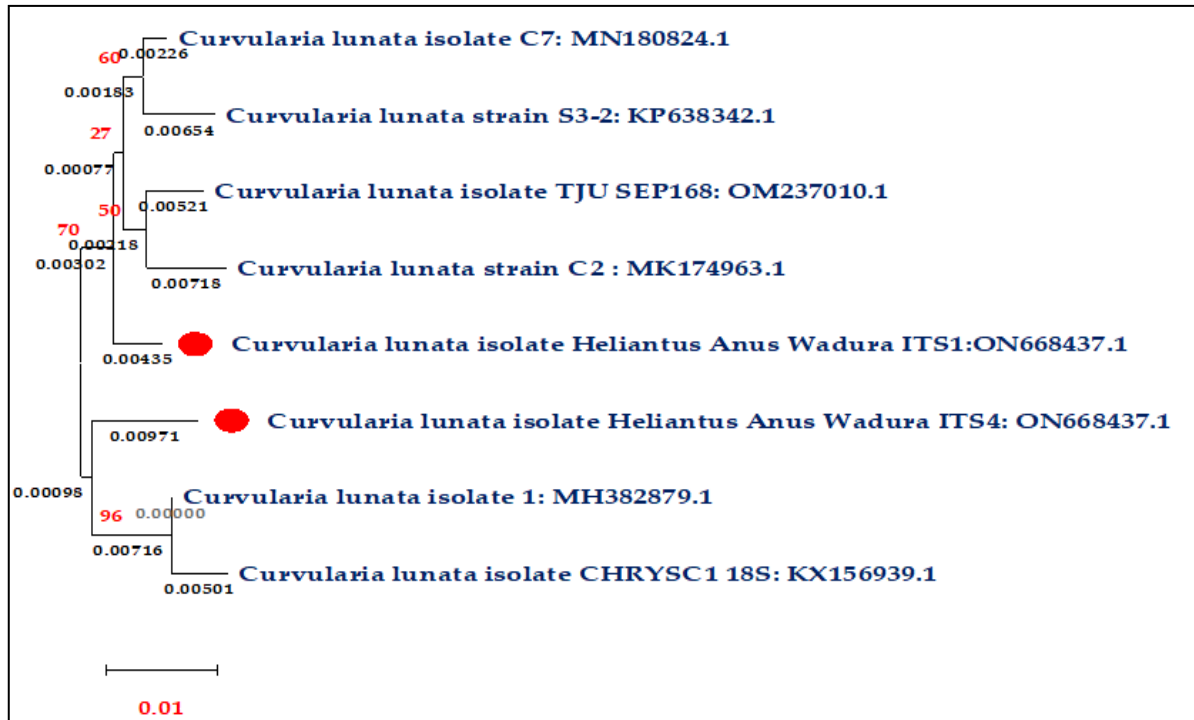


Fig. 3: Cluster diagramme showing the similarity of new isolate of *Curvularia lunata* with available sequences of similar pathogen species.

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