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NEW DISEASE REPORT

First report of *Fusarium incarnatum* causing leaf spot of fenugreek in Iran

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KEYWORDS

Trigonella foenum-graecum

Fenugreek (*Trigonella foenum-graecum*) is an annual herbaceous plant belonging to the Fabaceae family. In Iran, fenugreek is an important edible and medicinal leafy vegetable with a long history of cultivation. In December 2024, a leaf spot disease was observed on five fenugreek farms in Hormozgan province, Iran. The disease incidence varied from 5 to 20% amongst the farms. The spots were circular, 5–10 mm in diameter, and dark brown to black in colour which developed over the leaf surface and caused blight symptoms (Fig. 1).

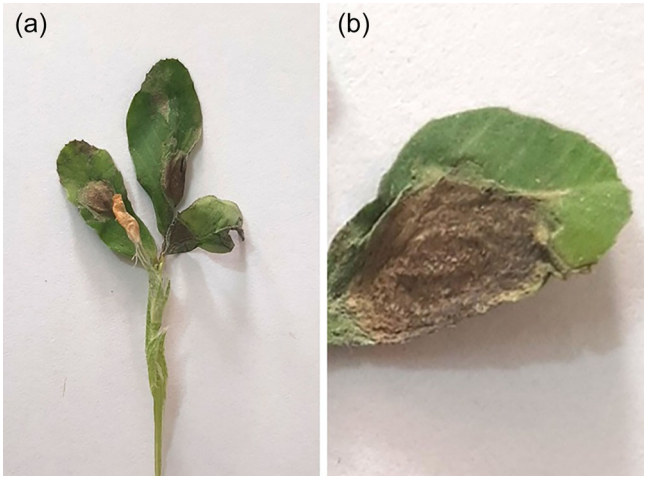


FIGURE 1 a) Dark brown to black spots on fenugreek leaves on a plant collected from the field; b) Close-up of the spot

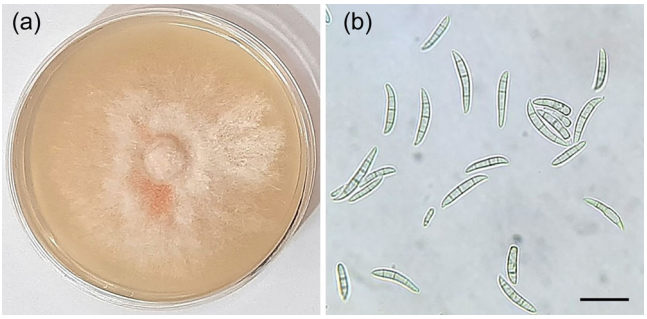


FIGURE 2 a) Seven-day-old colony of *Fusarium incarnatum* FLS14 isolated from fenugreek leaves with spot symptoms; b) Macroconidia. Bar = 10 μm

To determine the causal agent, leaf pieces of 5 × 5 mm were taken from the border between healthy and necrotic tissues, surface sterilised with 1% sodium hypochlorite for five minutes, rinsed thrice with sterile distilled water, placed on potato dextrose agar (PDA) and incubated at 27°C for one week. *Fusarium* colonies were predominantly isolated from the pieces. On PDA, the colonies grew rapidly and produced abundant dense aerial mycelia that initially were off-white and became beige with age. Light orange sporodochia were produced by some isolates. Macroconidia were relatively slender with a curved dorsal surface and a straighter ventral surface, measuring 10–14 μm (Fig. 2) (Leslie & Summerell, 2006).

Total genomic DNA of three representative isolates (FLS14, FLS24 and FLS84) was extracted from seven-day-old cultures and ITS1/ITS4, EF1/EF2 and 5f2/7cr primers were used for PCR amplification and

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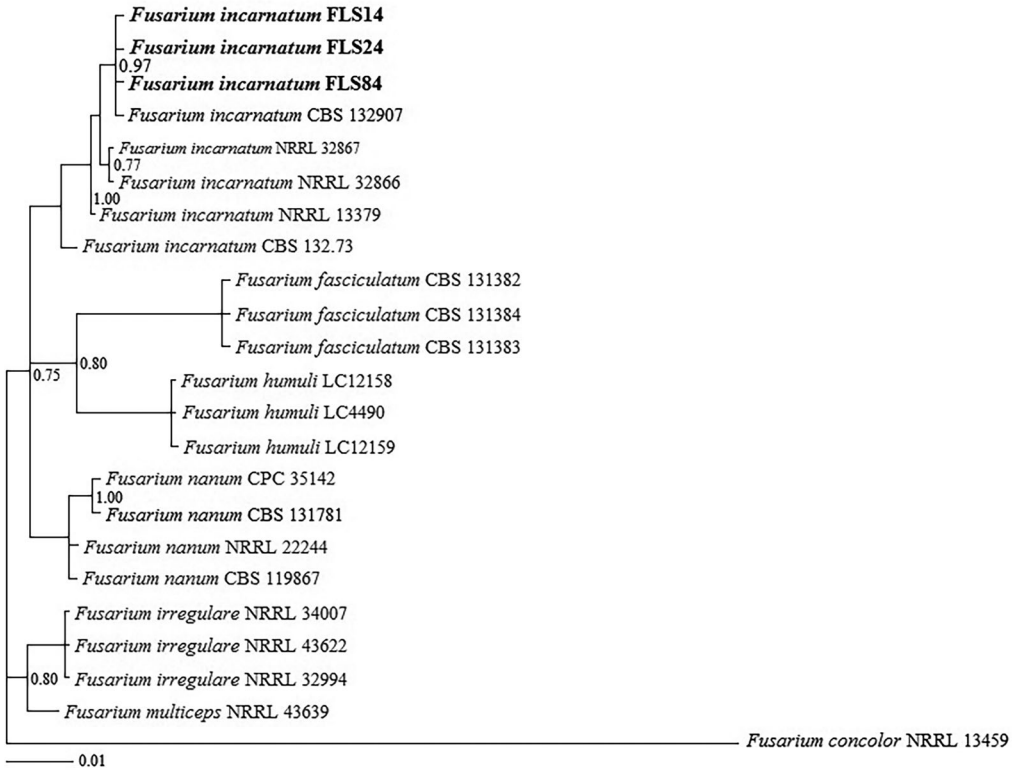


FIGURE 3 Phylogeny of *Fusarium incarnatum* isolates inferred from Bayesian analysis using combined sequence data of the ITS region, *tef-1α* and *rpb2* genes. Sequences of isolates FLS14, FLS24 and FLS84 obtained in this study are indicated in bold. Support values (posterior probability values) are indicated at the branches. The tree is rooted to *Fusarium concolor* NRRL 13459.



FIGURE 4 a-c) Dark leaf spots on fenugreek seedlings inoculated with *Fusarium incarnatum* isolates; d) Control plant

Sanger sequencing of the internal transcribed spacer region (ITS1–5.8S-ITS2), translation elongation factor-1 alpha gene (*tef-1α*) and RNA polymerase second largest subunit (*rpb2*) (White et al., 1990; O'Donnell et al., 1998; Lombard et al., 2019). The ITS (Genbank Accession No. PQ897996, PQ897997 and PQ897998), *tef-1α* (PQ902079, PQ902080 and PQ902081) and *rpb2* (PV602127, PV602128 and PV602129) sequences had 99–100% identity to *F. incarnatum* strains in GenBank (GQ505769, MN170410, GQ505793 and GQ505794). The phylogenetic analyses of the aligned sequences were carried out using the software MrBayes v. 3.2.1. Based on Bayesian inference of combined ITS, *tef-1α* and *rpb2* sequences, the isolates obtained in this study were identified as *F. incarnatum* (Fig. 3).

To confirm pathogenicity, 30 healthy 25-day-old fenugreek seedlings grown in 15-cm diameter plastic pots containing an autoclaved peat/soil mixture were inoculated through a foliar spray application with conidial suspensions (1×10^6 conidia ml^{-1}) prepared

from the *F. incarnatum* isolates. Control plants were treated with sterile distilled water. All plants were kept in a greenhouse at 30 ±2°C with a relative humidity of 55%. The experiments were conducted twice with four replicates. Inoculation led to the appearance of small, brown lesions, measuring 2–5 mm in diameter which became more widespread and coalesced with each other. No disease symptoms appeared on the leaves of the control seedlings (Fig. 4). *Fusarium incarnatum* was re-isolated only from symptomatic leaves.

Recent reports describe *F. incarnatum* infecting foxtail millet and muskmelon in China (Cao et al., 2019; Zheng et al., 2019) and *Cucumis trigonus* in India (Mali et al., 2015). To our knowledge, this is the first confirmed report of *F. incarnatum* on fenugreek worldwide.

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