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Detection of tomato anthracnose caused by *Colletotrichum truncatum* in India

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Abstract Tomato fruits with typical anthracnose symptoms of small, sunken, dark brown lesions were observed in the farmers fields in Jalna, Maharashtra, India. Based on morphological and molecular characterization, the fungus was identified as *Colletotrichum truncatum*, which is reported for the first time causing anthracnose on tomato in India.

Keywords *Solanum lycopersicum*

Tomato, *Solanum lycopersicum,* is one of the most important vegetable/fruit crops of India. Anthracnose (fruit rot) of tomato caused by *Colletotrichum* species is an important disease worldwide, resulting in post-harvest fruit decay, which severely affects marketability of the fruits. In August 2016, tomato fruits (5%) showing typical anthracnose symptoms of small, sunken, dark brown lesions were observed in two different fields in Jalna, Maharashtra (Fig. 1). Five tomato fruits showing typical anthracnose symptoms were collected and transported to a laboratory at Mahyco research centre, Jalna for study.

Tomato fruits showing anthracnose symptoms were surface sterilized with 1% NaOCl for 2 min and then rinsed twice with sterile distilled water. Surface sterilized sunken necrotic tissue of tomato was examined under a microscope. Twenty μl of sterile distilled water was dispensed onto each visible acervulus. Conidia were pipetted out and their presence was checked under a microscope. Serial dilutions of conidial suspension were made by adding sterile distilled water until a spore concentration of 1 or 2 spores per 10 μl was achieved. Ten μl of conidial suspension was pipetted into each of six sectors on the surface of potato dextrose agar (PDA) containing 50 mg/l streptomycin sulphate in 9-cm-diameter petri dishes and allowed to dry in laminar air flow cabinet for 5 min. Plates were then incubated at 28 °C, with 16/8 h light/dark cycle, respectively, for 3 to 4 days and a pure culture of the *Colletotrichum* isolate was obtained by sub-culturing on fresh PDA plates. Initial identification of all three pure cultures of the *Colletotrichum* isolates was done on the basis of morphological characteristics of conidia. Colonies of the *Colletotrichum* isolate were grey colored and had a mean growth rate on PDA at 28 °C and a 16 h light/8 h dark

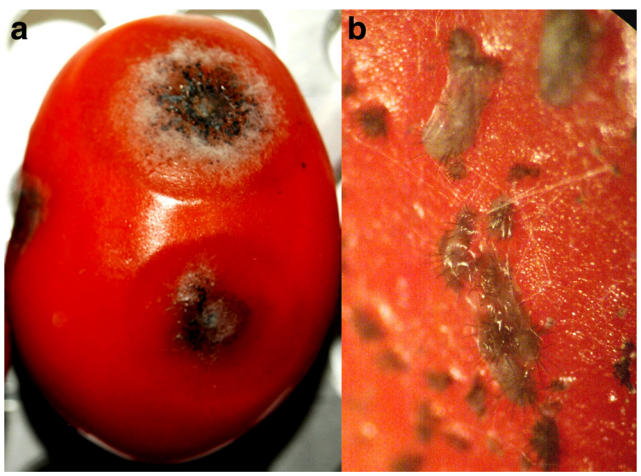

Fig. 1 **a** anthracnose symptoms on tomato fruit; **b** acervuli

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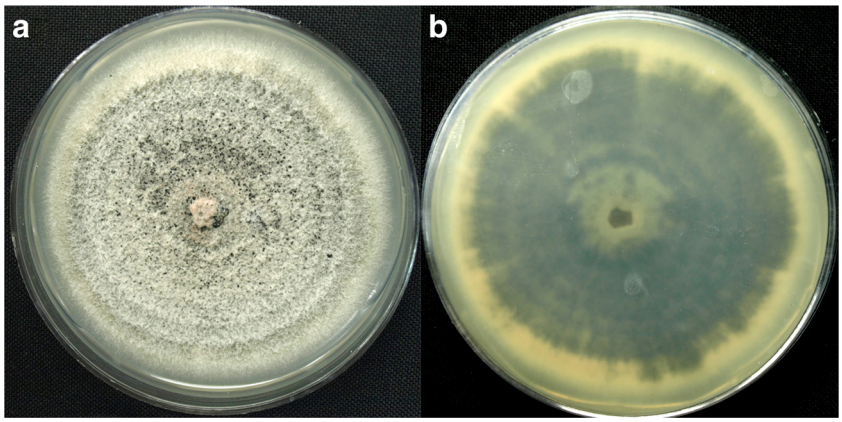

Fig. 2 *Colletotrichum truncatum* on PDA, ten days post inoculation, (**a**) from above; (**b**) from below

photoperiod of 8 mm/day. Acervuli were scattered across the colonies and contained dark grey conidial masses and dark setae (Fig. 2). Conidia were aseptate, hyaline, thin walled, falcate and 24.0–29.4 × 3.9–4.9 µm ($\bar{x}$ = 26.4 ± 1.3 × 4.5 ± 0.3 µm, n = 20). The morphological characteristics of this fungal isolate matched with the description of *Colletotrichum truncatum* (Damm et al. 2009).

PCR amplification of ITS rDNA, using the universal primer pair ITS 4/5 (ITS region of the nuclear ITS1–5.8S - ITS2 rDNA; White et al. 1990) and partial β-tubulin gene using Bt2a/b primers (Glass and Donaldson 1995) was conducted for molecular characterization of the *Colletotrichum* isolate. Blast searches in the NCBI database revealed that ITS and β-tubulin gene sequences (Accession Nos KY399773 and KY399774, respectively) had 99% and 100% similarity to *C. truncatum* (KX197395 and KP823848, respectively). Based on phylogenetic analyses and morphological characteristics, the *Colletotrichum* isolate obtained from infected tomato fruit was confirmed as *C. truncatum*. A pure culture of *C. truncatum* has been deposited in the National Fungal Culture Collection of India (NFCCI), Agharkar Research Institute, Pune, India (Accession No.4127).

To confirm pathogenicity of *C. truncatum* isolate 4127, ten fruits of each tomato cv. *Arka saurabh* and chilli (*Capsicum annum* cv. *Phule jyoti)* were obtained from plants raised in a green house. Fruits were first washed with sterile distilled water and then surface sterilised with 70% ethanol for 30 s. Tomato and chilli fruits were then pin pricked with a sterile syringe. Five fruits of each tomato and chilli were inoculated on pin pricks with 10 µl of *C. truncatum* isolate 4127 spores

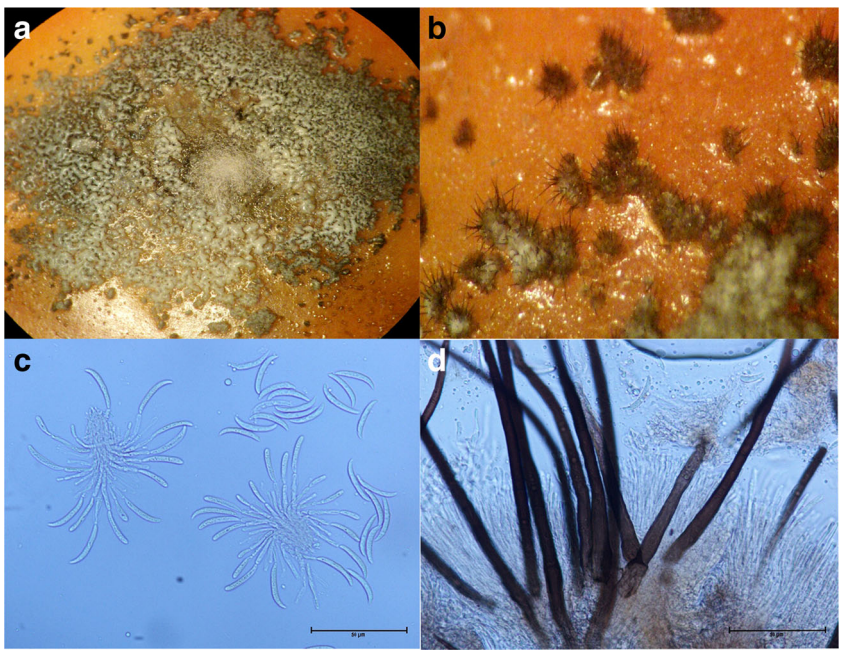

Fig. 3 **a** development of anthracnose symptoms on tomato fruit following artificial inoculation of *Colletotrichum truncatum;* **b** acervuli; **c** conidiophores with conidia; **d** setae. Bars: c-d = 50 µm

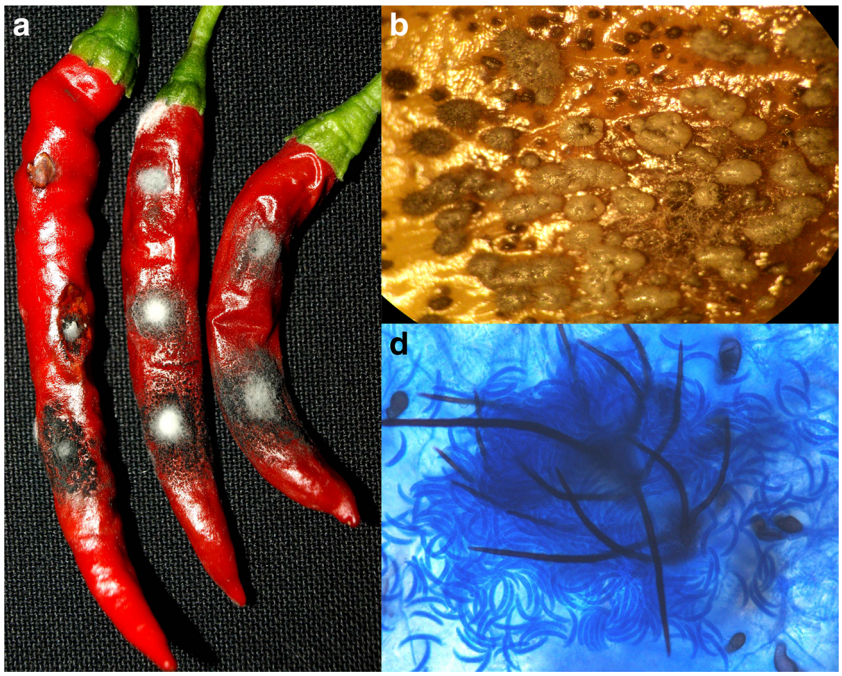

Fig. 4 **a** development of anthracnose symptoms on chilli fruit following artificial inoculation of *Colletotrichum truncatum;* **b** acervuli; **c** setae. Bar: c = 50 μm

(c.10^5 conidia/ml), obtained from the PDA culture plate while 5 fruits were inoculated with sterile water and used as controls. Inoculated tomato and chilli fruits were incubated in a chamber at 28 °C in dark with 90% humidity. After seven days, typical anthracnose symptoms developed on inoculated tomato (Fig. 3) and chilli fruits (Fig. 4). Pathogenicity experiment was repeated three times. Conidia were re-isolated from these diseased inoculated tomato and chilli fruits and observed under a microscope. Colony morphology, conidial measurements and sequences were identical to the original inoculated *C. truncatum* isolate 4127, thereby fulfilling Koch's postulates.

C. truncatum as a causal agent of chilli anthracnose has been reported in India (Chethana et al. 2015) and is reported to cause tomato anthracnose in China (Diao et al. 2014). To our knowledge, this is the first report of tomato anthracnose caused by *C. truncatum* in India.

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