Alternaria tenuissima causes leaf spot in makhana

Makhana (*Euryale ferox* Salisb), also known as 'fox nut' or 'gorgon nut' belongs to the family Nymphaeaceae. It is a floating annual aquatic plant with large leaves and prickly petioles, and grows in stagnant water bodies generally around 1.5 m deep, occurring within a wetland ecosystem. Tropical and subtropical climatic conditions favour the growth of this cash crop. Bihar, Assam, West Bengal and Odisha are some of the states in India where it is grown commercially as a high-value commodity[1]. It is cultivated in an area of about 20,000 ha, and Bihar contributes about 80% acreage and more than 90% production[2]. It has nutritional, medicinal and ritualistic significance in addition to a means of livelihood for thousands of fishing families; it also supports the cottage industry. Makhana is less prone to diseases than that of other crops; however, during a survey and surveillance conducted in April 2018, in Koshi region, Bihar, severe foliage infection was observed in makhana. The disease was widespread, occupying 25–30% of leaf area in about 20% plants in different ponds. Initially small, necrotic, light yellow lesions were discernible on infected leaves which later turned into sunken, light tan to brown spots, usually with concentric ring or target board pattern, and a yellow halo. Eventually, the spots coalesced, and these necrotic lesions expanded and joined together to cover the entire leaf (Figure 1). Seeds produced by infected plants were grey to black in colour, wrinkled, small in size and had low viability. The pathogen was isolated by surface disinfecting small fragments of symptomatic leaf tissues in 0.5% NaOCl, double-rinsing in sterile water, and plating onto potato dextrose agar (PDA) amended with 0.05 gl^{-1} streptomycin sulphate. The plate was incubated at 28° ± 1°C with a 12-h photoperiod. A fungus, resembling *Alternaria tenuissima* (Nees) Wiltshire was consistently isolated and preliminarily identified based on morphological characteristics. The fungus was slow-growing, producing effuse, olivaceous colony that turned dark grey to black having white margins and branched, septate, brown mycelium (Figure 2 *b*). Conidiophores were branched at an acute angle, straight or flexuous, septate and light pale in colour, and smooth-walled (Figure 2 *c*). Conidia (n = 30) were 10–45 µm long (avg 27.6; SD ± 3.9 µm), 6–11 µm wide in the broadest part (avg 8.2; SD ± 2.3 µm) with a beak 1.5–6.0 µm long (avg 3.2; SD ± 2.4 µm), and 2–6 transverse and

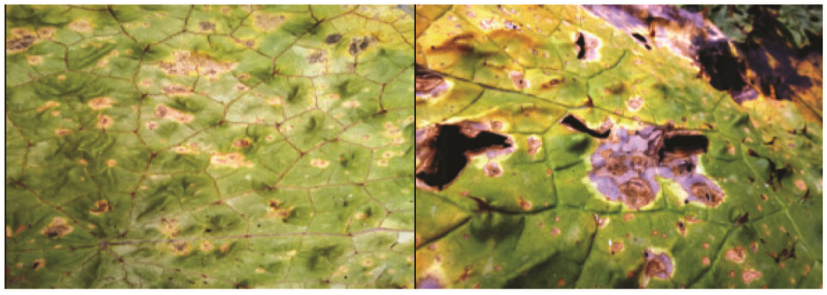

Figure 1. Symptoms of leaf spots caused by *Alternaria tenuissima* in makhana.

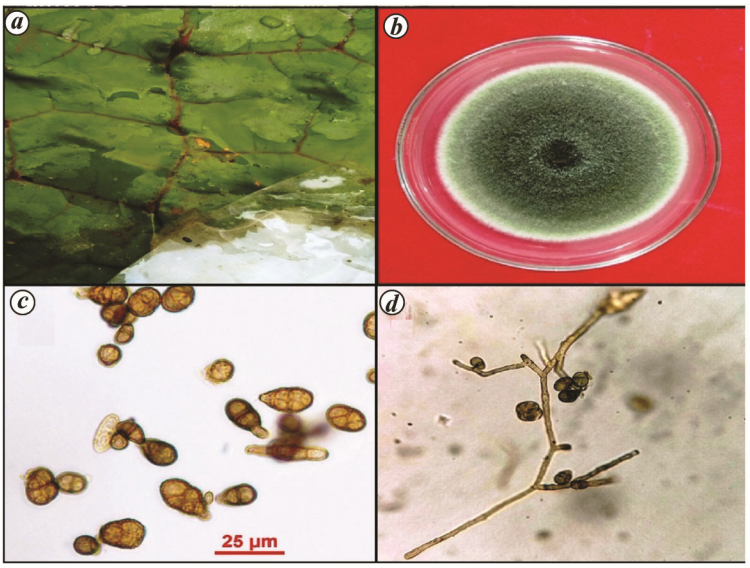

Figure 2. ***a***, Developed leaf spot on inoculated test leaf of makhana in pathogenicity test; ***b***, Pure culture of *Alternaria tenuissima* on potato dextrose agar; ***c***, Conidia of *A. tenuissima* (40×); ***d***, Conidiophore branching at acute angle (10×).

0–2 longitudinal septa (Figure 2 *d*). These morphological characteristics and measurements were similar to those of *A. tenuissima*. This was further confirmed by Indian Type Culture Collection (ITCC), Indian Agricultural Research Institute, New Delhi (ID 10.826.18). The molecular identification was done by sequencing the internal transcribed spacer (ITS) region of rDNA using primers ITS1 and ITS4 (ref. 3). The generated sequences were submitted to GenBank (accession no. MH938072). Mega-BLAST analysis of sequence showed more than 99% homology with *A. tenuissima* strains MH032750 and MH864067. To fulfil Koch's postulates, conidial suspensions (5×10^5 conidia ml^{-1}) from cultures on PDA were inoculated on ten leaves of 35-day-old *Euryale ferox* plants by pin-prick method[4]. Conidial suspension was injected using modified hypodermic needle at the prick point and the leaves were covered by moist sterilized cotton swab along with sterilized aluminium foil. After five days of inoculation, the aluminium foil was removed and test leaves were exposed to natural humidity regime. Ten leaves of *E. ferox* plants were inoculated similarly with sterilized distilled water. The tested leaves were continuously monitored for the appearance of symptoms. After two weeks, typical symptoms of brown spots with concentric ring were observed on all the inoculated leaves (Figure 2 *a*), whereas the control leaves remained asymptomatic. *A. tenuissima* was specifically reisolated and identified from the inoculated symptomatic leaves, thus confirming Koch's postulates. Thus the occurrence of leaf spot on *E. ferox* may pose a serious threat to the cultivation of makhana and result in higher yield reduction.

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Bt-cotton hybrid seeds and 'refuge-in-bag' strategy

This is in response to the correspondence by Muralimohan and Mahesh[1], wherein the authors have conducted the much-needed *Bt*-trait quality analysis of dual *Bt*-gene Bollgard II™ (BG II) cotton seeds sampled from the markets of central and southern India. Their contention that the *Bt* cotton seed producers, now, need not 'purposefully mix non-*Bt* seeds' to implement 'refuge-in-bag' (RIB) because by default the proportion of non-*Bt* refuge seeds was ~5% in 93% of the BG II seeds sampled, is not correct, for the following reasons.

The Ministry of Agriculture and Farmers Welfare, Government of India, has directed that all BG II cotton seed producers should completely switch over to RIB mode of refuge delivery from the *kharif* of 2020. Accordingly, the seed producers have suitably geared-up their seed production and packaging processes to achieve this objective. Simultaneous implementation of the new seed packaging process, across all *Bt*-seed producers, would henceforth ensure correct blend of non-*Bt* refuge seeds (5–10%) with trait-validated BG II seeds (90–95%). It is hoped that the in-built quality assurance in the new packaging system would bring uniformity across all brands of BG II in terms of refuge quality and proportion in the blend, which appears to be lacking now. The current situation is reflected in the authors'[1] analyses which reveals a wide variation in *Bt*-trait levels in the sampled bags, ranging from 61% to >95%. In addition, the constituent *Bt* traits (Cry1Ac, Cry2Ab2) were found singly in about 1% of the seed samples. Hence, the BG II seed producers should implement RIB for BG II which would entail stewardship and quality management of the highest order. Here is an opportunity for the *Bt* cotton seed producers to correct the existing inadequacies in refuge proportion and quality[2].

The quality of non-*Bt* refuge seeds (supplied in a separate packet and which these authors[1] have not analysed) is a big concern. Kranthi *et al.*[3] have shown that refuge seeds often contained *Bt* traits and the refuge plants varied widely in phenotype, viz. bloom initiation, bloom period, fibre quality and yield from that of the BG II hybrid. The refuge plants should be isogenic (same parentage but lack *Bt* trait) or close hybrids with matching phenotype, enabling the farmer to get a good yield in the absence of pest incidence. In short, all the above anomalies can be set right with the new RIB system.

The authors[1] also conclude that the RIB option may not be effective in delaying evolution of *Bt* resistance in bollworms, because in their view the default presence of ~5% non-*Bt* seeds along with *Bt* seeds from the time of commercialization of BG II in 2006 did not delay the evolution of *Bt* resistance in pink bollworm (PBW).

We need to get to the basics to understand this issue. PBW is currently resistant to both toxins expressed by BG II. PBW can multiply only on conventional cotton and no other host, implying that the selection pressure to evolve *Bt* resistance would have been the greatest in this pest relative to another major bollworm like *Helicoverpa armigera*, which can multiple on more than a dozen host crops (natural refuge). Thus, it is critical to supplement refuge (structured/RIB) with integrated pest management (IPM) measures for the management of PBW. Structured refuge and IPM for *Bt*-cotton were neglected by the farmers and consequently resistance evolved. Right from the beginning, the *Bt*-technology