New Disease Reports

Pythium deliense, a pathogen causing yellowing and wilt of black pepper in India

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Black pepper (*Piper nigrum*) is cultivated for its fruit, commonly known as a peppercorn, and is one of the most exported spice crops in the world. Soil-borne diseases like foot rot caused by *Phytophthora capsici* and slow decline caused by combined infections of *P. capsici* and nematodes, *viz. Meloidogyne incognita* and *Radopholus similis,* are the major threats for the crop. During the post-monsoon period of 2014 and onwards, black pepper vines in all the major growing areas of Kerala and Karnataka, and especially in the districts of Idukki, Kasaragod, Kozhikode, Malappuram, Palakkad and Wayanad (Kerala) and Kodagu (Karnataka), turned yellow and later were found drying up (Fig. 1). Analysis of root and rhizosphere soil from the affected plants resulted in the isolation of a *Pythium* sp.

Examination of *Pythium* isolates revealed filamentous inflated/torulated sporangia similar to *P. aphanidermatum* but differed by the bending of oogonial stalks towards the antheridia (Fig. 2). The smooth oogonia, aplerotic oospores, broad apical intercalary antheridia resembled *Pythium deliense* (van der Plaats-Niterink, 1981).The main hyphae measured 7-8 µm. Sporangia were mostly terminal with swollen side branches and encysted zoospores measured 5.0-9.0 µm in diameter. Oogonia were smooth, terminal and globose, 18.3-20.5 µm diameter. Antheridia cells measured 6.5-8.1 µm. Oospores were aplerotic, 15.4-19.7 µm in diameter and wall thickness ranged from 1.5 to 2.4 µm. A total of 20 rhizosphere soil samples and 10 root samples from yellowing black pepper vines (cvs. Panniyur-1, Pannniyur-4 and IISR-Girimunda) from the Kozhikode and Wayanad districts were collected and *P. deliense* was isolated from 15 (75%) soil and 5 (50%) root samples.

The isolates were exposed to different temperatures and pH. They could grow at a pH range of 4.5-10 (Fig. 3a) and at a temperature range of 15-40°C with an optimum temperature of 28 to 32°C (Fig. 3b). Pathogenicity was tested on different parts of black pepper vines by inoculating mycelial plugs of an actively growing culture on detached and intact plants. The plants were kept at a temperature of 25-28°C, 75-80% relative humidity with a 12 hour photoperiod. Plants showed infection within 24 hours on leaves and collar and root infection in 9-15 days. The pathogen was re-isolated fulfilling Koch's postulates (Fig. 4). *P. deliense* was also found pathogenic to roots of eight cultivated varieties of black pepper (Table 1).

DNA from five isolates was amplified by PCR using ITS1 and ITS4 primers (Al-Sa'di *et al*., 2007). Amplicons of the expected size were sequenced a BLAST search showed 99% identity with *P. deliense.* DNA sequences were deposited in GenBank (Accession Nos. MH017855, MH017856, MK416215, MK416216 and MN197462).

There are previous reports of *Pythium* spp. being isolated from black pepper in India (Anandaraj *et al.,* 1991). A root disease of black pepper characterised by yellowing and wilting and caused by *P. splendens* was reported from Japan where they also identified *P. deliense* as a weak pathogen (Matsuda *et al.,* 1998). Lodhi *et al.* (2004) reported *P. deliense* from cultivated *Piper betel* in Pakistan. However, this is the first report of *P. deliense* from the rhizosphere and roots of black pepper from India. Thorough research is required to evaluate the impact of the pathogen on yellowing and wilting of black pepper.

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Figure 1

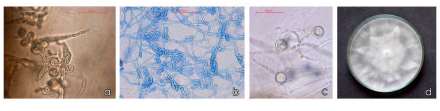

Figure 2

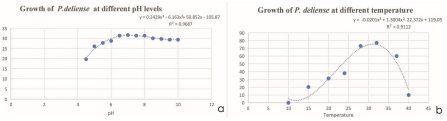

Figure 3

Figure 4

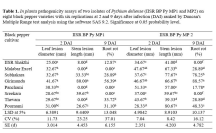

Figure 5

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