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Corynespora cassiicola causes leaf spot disease on lettuce (*Lactuca sativa*) cultivated in hydroponic systems in Thailand

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Abstract Lettuce (*Lactuca sativa*) is an annual vegetable that is widely cultivated in hydroponic systems in Thailand. In 2016, a leaf spot disease was commonly found on hydroponically-grown lettuce plants in glasshouses in Songkhla province, southern Thailand. The morphology of the fungus that was consistently isolated from the leaf spots was identical to that described for *Corynespora cassiicola*. The identity of the fungus was confirmed by sequencing the ITS and LSU gene regions. Koch's postulates were satisfied, proving that *C. cassiicola* was the causal agent of the recent outbreaks of leaf spot in hydroponically-grown lettuce in Thailand. This paper is the first report of the disease in this country.

Keywords *Corynespora* · Hydroponic · Leaf spot · Lettuce

Lettuce (*Lactuca sativa*) is the world's most popular leafy salad vegetable. In Thailand, the ready-to-eat lettuce cultivars Batavia (BV), Green oak (GO) and Red oak (RO) are widely cultivated in hydroponic systems. In these systems most crops are regularly sprayed or misted, a practice that can assist in the dispersal of propagules of plant pathogens and in the development of diseases, particularly in closed glasshouses. Koohakan et al. (2008) reported that the major diseases of lettuce grown in hydroponic systems in Thailand as root rot caused by *Pythium myriotylum*, bacterial rot caused by *Erwinia* sp. and *Pseudomonas* sp., collar black rot caused by *Rhizoctonia* sp., and leaf spot caused by *Cercospora* sp.

Between June and August 2016, a leaf spot was found on lettuce plants cultivated in hydroponic systems in three districts of Songkhla province in southern Thailand. The first symptoms appeared on the upper surfaces of leaves and the lesions later extended to the lower leaf surfaces. The spots on affected leaves of lettuce plants were brown to dark brown with light and dark concentric rings, circular to irregular, and 5–15 mm in diameter (Fig. 1a). The most severe infection was in young lettuce plants, where small spots increased in size, coalesced and eventually resulted in leaf blight and death of the plant (Fig. 1b). In individual glasshouses, the disease spread rapidly to most lettuce plants, causing losses in both quality and quantity.

Conidia and conidiophores were observed on infected leaves incubated for 48–60 h in a moist chamber. Conidiophores were mostly solitary, straight cylindrical, 94.5–162 × 2.7–4 μm, 2–20 septate, and dark brown (Fig.1d–f). The conidia, produced singly or in acropetal chains, were obclavate, cylindrical, straight or curved, 62–127 × 5–8 μm (mean 100 × 6.8 μm), with 5–20 cells separated by hyaline pseudoseptate, and dark brown to pale brown (Fig. 1d–f).

Small portions of leaves with a spot and surrounding healthy tissues were excised with a sterile razor blade, and were surface-sterilised by using NaOCl and 70% ethanol. The tissue samples were placed on potato dextrose agar (PDA) and incubated at room temperature (28 ± 2 °C) for 24 h. The small pieces of agar containing a single hyphal tip were cut and transferred to PDA slants for further use.

Fungal colonies grown on PDA for 10 days were brown to dark brown, formed concentric circular rings, and were

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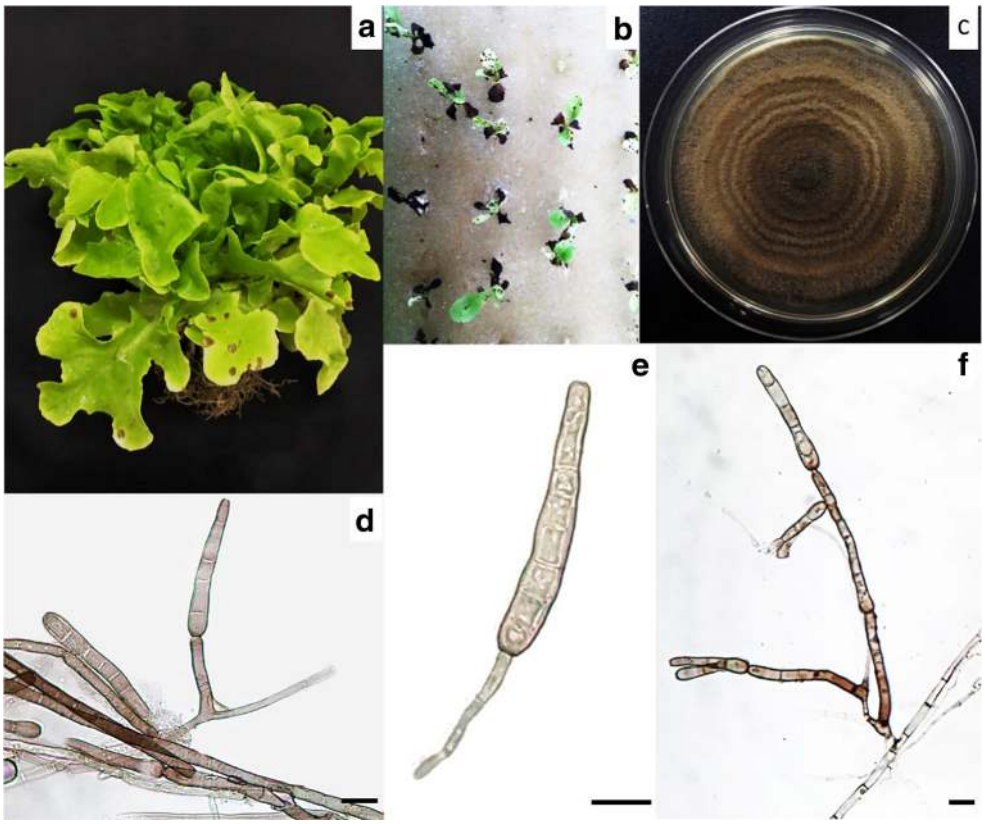

Fig. 1 *Corynespora* leaf spot on lettuce, (**a**) lesion on lettuce cultivated in hydroponic system, (**b**) severe disease on young lettuce seedlings, (**c**) a colony growing on PDA, (**d**) and (**e**) conidiophores with obclavate conidia, and (**f**) acropetal chains of conidia. The scale bars represent 10 μm

velvety with abundant aerial mycelia (Fig. 1c). All of the isolates produced a purplish-coloured exudate that diffused into the culture medium. The morphological characteristics of the fungus were consistent with those described for *Corynespora cassiicola* by Ellis and Holliday (1971). A culture of the fungal pathogen (SK01) was deposited in the Culture Collection of Pest Management Department, Faculty of Natural Resources, Prince of Songkla University (PSU), Thailand, with accession number PSU-PM-SK01.

To confirm the identity of the leaf spot pathogen morphologically identified as *Corynespora cassiicola*, an isolate (SK01) was grown on PDA at room temperature for 1 week, for DNA extraction. Fungal total DNA was extracted from the mycelium by the cetylmethyl ammonium bromide (CTAB) method (Manamgoda et al. 2012). The intertranscribed spacer (ITS) and large subunit (LSU) gene regions were amplified using BIO-RAD T100™ Thermal Cycler (BioRad, Hercules, CA, USA). Amplification of the gene region used the primer pair PN3/PN16 (Neuveglise et al. 1994) for ITS and LROR/LR (Vilgalys and Hester 1990) for LSU. The PCR was performed in 50 μl of reaction mixture containing 10 pmol of each primer, 2× Dreamtaq Green PCR Master Mix (Thermo Scientific) and 50 ng of DNA template. An initial denaturation step for 3 min at 95 °C was followed by 35 cycles of denaturation for 30 s at 95 °C, annealing for 30s at 50 °C, and extension for 1 min at 72 °C, with a final extension step of 10 min at 72 °C. The PCR products were visualised by agarose gel electrophoresis. The ITS and LSU gene regions were sequenced at Macrogen (Seoul, Korea) using the same primers as used in the PCR reaction. Using BLAST analyses, the sequences obtained were compared with known sequences available in Genbank (The National Center of Biological Information). The BLAST search revealed that the sequence of isolate SK01 had a 100% match with *C. cassiicola* E9807C (JN541214, *Malvaviscus concinnus*, Ecuador) for the ITS gene, and with *C. cassiicola* C13–1 (KF590123, unknown host, Japan) for the LSU gene. This sequence has been deposited in GenBank with accession number LC177365 for LSU and LC177366 for ITS.

Pathogenicity tests were conducted twice by spraying 14-day-old healthy lettuce plants each of Batavia, Green oak and Red oak cultivars with a spore suspension (1×10^6 spore/mL) prepared from the 10-day-old colonies. The control lettuce plants were sprayed with autoclaved distilled water instead of spore suspension. Then, all treatments were incubated in a moist chamber until disease symptoms appeared. For each inoculation, brown spots, similar to those occurring on the naturally infected plants, developed on the young healthy

lettuce plants within 48 h of the spray inoculation. The control lettuce plants remained healthy. *Corynespora cassiicola* was consistently isolated from the leaf spots on inoculated lettuce plants following the same method that was used for the initial isolations. Koch's postulates were therefore fulfilled. This paper is the first report of *C. cassiicola* causing a leaf spot on hydroponically-grown lettuce in Thailand.

There are several fungicides used to control *Corynespora* leaf spot. Chlorothalonil and Propane-1, 2-diol in combination with copper oxychloride were the best chemical agents to control *Corynespora* leaf spot of tomatoes (Kingsland and Sitterly 2008). Boscalid, previously known as carboxamides, inhibited mycelial growth of *C. cassiicola* by 100% after 4 days incubation (Miyamoto et al. 2009). However, chemical fungicides are potentially toxic to humans, can cause emissions to natural waters and may harm biodiversity. Biocontrol is an alternative environmentally friendly method to control plant diseases. For example, Thi et al. (2012) reported that a culture filtrate of *Trichoderma* sp. H921 inhibited spore germination of *C. cassiicola* close to 100% in vitro. Seung et al. (2013) found that the used of 200 ppm of iturin, an antifungal substance produced by *Bacillus amyloliquefaciens* CNU114001 showed potential to inhibit the mycelial growth of *C. cassiicola*. In addition, sanitation is the key to maintaining a disease-free hydroponic system. The solution tanks will have to be drained and all equipment must be cleaned with a bleach solution before planting subsequent crops (Kaiser and Ernst 2012). Thus, biocontrol and sanitation alternatives may be preferable choices for the control of leaf spot disease caused by *C. cassiicola* in lettuce cultivated in hydroponic systems, and these topics are here left for future studies.

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