

First Record of *Alternaria alternata* causing necrosis of Thuja (*Thuja occidentalis*) in Kazakhstan

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Thuja is one of the ornamental plants used for landscaping parks and health resorts. The plant is distinguished by a pyramidal and conical crown shape and the presence of many thin branches with scale-shaped needles, green all year round. In addition, this plant has a number of antimicrobial properties, which affects the popularity of the plant in landscaping the health resort territory (Bakht et al. 2020, Chindyaeva et al. 2020). In January 2020, symptomatic Thuja plants were observed in Southern Kazakhstan. Symptoms included distortion of the crown. External examination of the trees revealed the presence of white fluffy mycelium on Thuja branches. The branches acquired a yellow color with a necrotic lesion developing below the affected area. Samples of infected branches from different Thuja trees (n = 13) were collected. The infected branches were cut into small pieces (5 × 5 mm), washed in 70% ethanol for 30 min, and then rinsed three times with sterile distilled water. Later, these pieces were placed on Sabouraud's medium (Laboratorios Conda S.A., Spain) and incubated at 28°C for 7 days. Yellow-green

colonies grew from the pieces of wood. The colonies had a light gray-whitish aerial mycelium. Conidia (n = 35) were pale to dark brown in color, irregular and ellipsoid to ovoid conical in shape. The size of the conidia varied from 5 to 25 $\mu\text{m} \times 6$ to 12 μm (n = 40) with longitudinal and transverse septations. These morphological characters were previously described and corresponded to the *Alternaria alternata* (Simmons et al. 2007). Genomic DNA was extracted from mycelium using the liquid nitrogen and phenol-chloroform extraction method (Butler 2012). A 568 bp product of the Alt a1 gene and 472 bp product of the calmodulin protein-coding gene was amplified using following primer pairs Alt-for/Alt-rev (Hong et al. 2005) and CALDF1/CALDR1, respectively (Lawrence et al. 2013) (Integrated DNA Technologies, Inc., Coralville, IA, USA). The PCR reaction was done in a SimpliAmp thermal cycler (Applied biosystems, Waltham, MA, USA) under the following conditions: initial denaturation at 94 °C for 1 min, 35 cycles at 94°C for 30 s to denature, 57°C for 1 min for annealing, and 72°C for 1 min for extension. A final extension step at 72°C for 10 min was also included. The sequencing was done using BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) and the sequence was deposited in GenBank with accession no. OL542696 (Alt al) and OM471502 (calmodulin). These sequences were compared with other sequences in the GenBank by using the BLAST analysis (MZ222274.1 and MN473132.1). The phylogenetic analysis was carried out with MEGA 6 software (The Pennsylvania State University, University Park, PA, USA). To confirm the pathogenicity, 10 thuja branches from healthy trees from another area without visible pathologies were inoculated with a suspension of conidia (100 conidia/ml; obtained from 2-week-old cultures). Control samples were inoculated with sterile distilled water. The inoculated branches were placed in sterile plastic containers to maintain high humidity and incubated for 10 days at 28°C. After 7 days, irregular shaped lesions and fungal growth was observed at the site of

inoculation. The affected area gradually increased in size with similar symptomatology to that described above. Re-isolation of the pathogen and identification based on morphological features and sequencing confirmed the presence of the *A. alternata* pathogen.

To our knowledge, this is the first report of *A. alternata* causing branches of thuja in Kazakhstan. Thuja is a rare plant species for this region; the cost and care are expensive. This case will allow timely diagnosis of the disease caused by *Alternaria spp.* in the future. It is necessary to develop preventive measures and a protocol for the treatment of thuja from a fungal infection.

The author(s) declare no conflict of interest.

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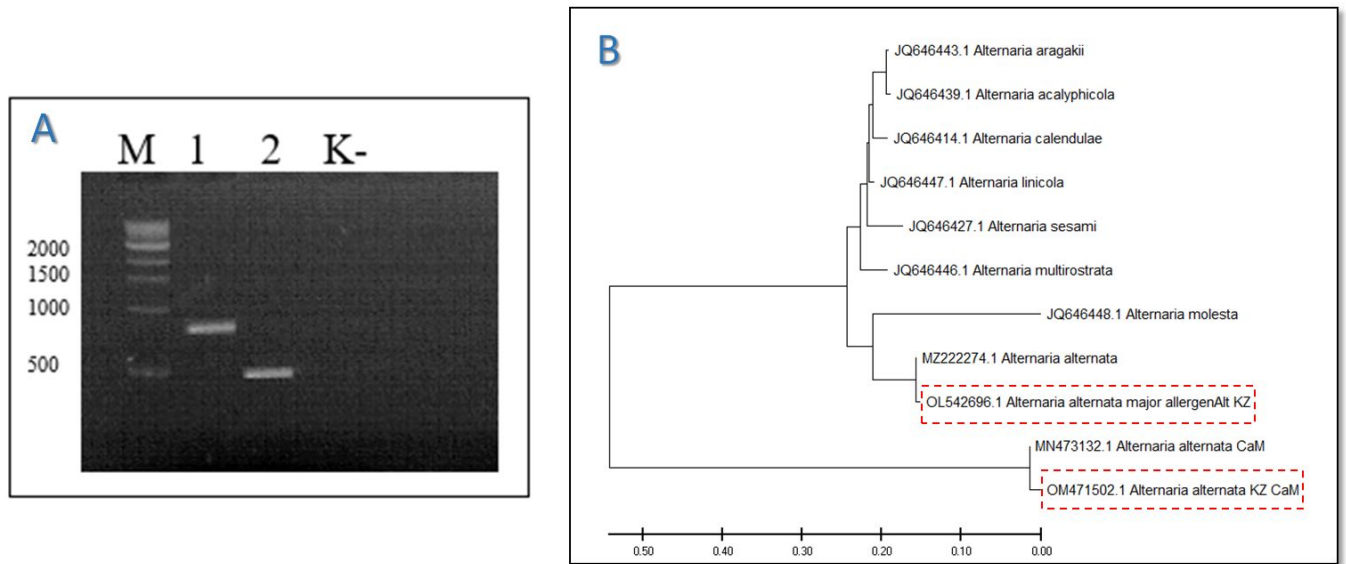
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74 Supplementary Figure S1 shows thuja branch severely attacked by *Alternaria alternata* collected
75 from infected tree and its morphological characteristics.

76 Supplementary Figure S2 shows the results of PCR analysis with *Alt* and calmodulin primers and
77 a phylogenetic tree.



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2 Figure S1. Thuja branch affected by *Alternaria alternata* (A); Morphological characteristics of
3 *A. alternata*: colonies after 7 days at 28°C on Sabouraud agar (B) and microscopic characters of
4 *A. alternata* (conidia and hyphae) (C).



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2 Figure S2. PCR products of specific primer pairs and DNA isolated from *Alternaria alternata*

3 strain (A): lane 1, DNA ladder (bp); lane 2, primer pair of calmodulin; line 3, primer pair of Alt

4 al; Phylogenetic analysis (tree) generated with using Alt a1 (484 bp) and calmodulin (480 bp)

5 genes (B).