First report of *Sclerotinia sclerotiorum* on watercress (*Nasturtium officinale*) in aquaponic system in Hungary

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Watercress (*Nasturtium officinale*) is an aquatic dicotyledonous vegetable belonging to *Brassicaceae* (Aiton 1812). Watercress was grown in an aquaponic system on fired clay ball medium at the Aquaponic Research Station of the University of Debrecen, in the city of Debrecen (Hungary). During January 2020, 3-month-old plants showed symptoms in aquaponic cultivation. A visual survey showed 30% of plants with symptoms. Leaves and stems withered and showed white cotton-like mycelium. Mycelia from infected plants were placed on potato dextrose agar (PDA) and incubated at 25°C for seven days. Single hyphal tips were transferred to produce a pure culture. All ten fungal isolates showed similar morphological characteristics on PDA. Colonies consisted of white mycelia after three days and globoid to irregular and black 2.5 to 7 (average, 3) mm (n = 100 from ten plates) sclerotia formed ten days later, which are the typical morphological features of *Sclerotinia sclerotiorum* (Mordue et al. 1976). Molecular identification was performed with one of the ten isolates (Scl_B). Mycelia were grown in 250 ml of potato dextrose broth in a rotary shaker at 175 rpm at 24°C for six days. DNA was extracted from mycelium using a Nucleospin plant II (Macherey-Nagel, Germany) according to the manufacturer's protocol. PCR amplification (Kim et al. 2014) was performed with primers ITS1/ITS4 for the internal transcribed spacer region (White et al. 1990) on a Primus 96 thermal cycler (MWG Biotech, Germany). Specific polymerase chain reaction was performed with primers SSasprF/SSasprR (Abd-Elmagid et al. 2013). PCR products were sequenced by Microsynth Austria GmbH. NCBI BLAST analysis of the 440-bp ITS sequence (Genbank MW012403.1) showed 100% identity with the sequence of *S. sclerotiorum* (MT177267.1, etc.). The 170-bp specific gene sequence (Genbank MW959042.1) had a 100% similarity to hypothetical proteins (Genbank MK028159.1), with a 99.4% similarity to a portion of the S. sclerotiorum aspartyl protease gene (AF271387.1). Pathogenicity tests were carried out by inoculating surface-disinfested, 30-day-old watercress plants in plastic pots (15x15x12 cm). In three repeated experiments 90 watercress plants were measured. 15 plants (one plant per pot) were planted into the five-times autoclaved substrate (Biorgmix: pH 6.1±0.5%, N:1.5%, P_2O_5:0.7%, K_2O:0.5%, organic matter content:50%) and inoculated by ten wheat kernels that were colonized by *S. sclerotiorum* (Scl_B) (Garibaldi et al. 2019). 15 plants were planted into the substrate with ten non-inoculated kernels as a control. Plants were kept in an MLR-352 climatic test chamber (PHCbi, Japan) at 21 ± 1°C for 12 hr light:dark cycle. On the first day of the experiment complex nutrient solution (Tek-Land: N:5%, P_2O_5:5%, K_2O:5%, B:0.01%,

Cu:0,01%, Mn:0.02%, Mo:0.002%, Zn:0.016%) was used, then autoclaved water daily. Eight days later white mycelium appeared on every inoculated plant and five days later dark sclerotia formed on the stems. Based on the morphological characteristics the re-isolated pathogen was *S. sclerotiorum*. Similar results were detected in three repeated experiments with white mold fungus being reisolated from all 45 infected watercress plants. The 45 non-inoculated plants did not show any symptoms and any diseases. This pathogen has already been reported on watercress in the field (Farr et al. 1989; Boland and Hall 1994; Garibaldi et al. 2019). This is the first reported case of white mold on watercress in aquaponic system in Hungary.

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The publication is supported by the EFOP-3.6.1-16-2016-00022 project. The project is co-financed by the European Union and the European Union and the European Social Fund.

Sclerotinia sclerotiorum symptoms (observe discoloration of the stem and leaves) and signs (sclerotia) on watercress in growing in a aquaponic system

472x975mm (72 x 72 DPI)

Ten day-old culture on potato dextrose agar showing white mycelium and black sclerotia. Sclerotia average 3mm diameter for this Sclerotinia sclerotiorum grown at 25°C

693x714mm (72 x 72 DPI)