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BOTRYTIS CINEREA AS CAUSAL AGENT OF SUNFLOWER SEED GREY MOULD

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Abstract

Sunflower (*Helianthus annuus* L.) is one of the most important oil crops worldwide, including Serbia, where it is grown on about 200,000 ha with an average yield of 3 t/ha. *Botrytis cinerea* Pers. (1794) regularly occurs on sunflower seeds associated with the phenology of the host plant. However, sunflower seed is often affected by the *Rhizopus* spp., which causes similar symptoms on seed and seedlings as *B. cinerea*, and it is important to reliably differentiate those species based on morphological and molecular characteristics. The method used in this study for the detection of *B. cinerea* is described by ISTA method 7-003. The main task of this study was to isolate the causal agent of grey mould and rot of sunflower seed and seedlings. Infected seeds were covered with overgrown dark-grey mycelia with sporulation. In order to isolate the pathogen, the infected seed was transferred onto potato dextrose agar (PDA) and incubated for 7 days at 20°C. For morphological identification, 11 isolates were single-spored and subcultured onto PDA. Strains formed straight, noddy conidiophores, branched at the top and resembling a cluster with conidia. Conidia were ovoid or elliptical avg. 11.6 × 7.5 µm in size. Formed sclerotia were dark, irregular, gathering as large irregular or globular groups. Based on morphological characteristics isolated fungi were identified as *Botrytis cinerea*. Pathogenicity was confirmed using the *in vitro* agar slant method in the test tube with amended PDA. The pathogen was successfully reisolated and found to be morphologically identical to the original isolates, fulfilling Koch's postulates. Molecular identification was confirmed by PCR and sequencing of the transcribed spacer (ITS) region using ITS1/ITS4 primers. BLAST analysis of the obtained sequence of sample 27Sun (GenBank Acc. No. MH496033) showed 100% nucleotide similarity to sequences of six *Botrytis cinerea* isolates originating from Mexico (MH458873, MH458876, MH458881), isolate MG209662 from the USA and isolate MF741314 from Korea. The results of this study confirmed that *B. cinerea* was the causal agent of grey mould in the untreated sunflower seed.

Keywords: *Botrytis cinerea*, sunflower seed, molecular identification, ITS, sequencing

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