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Bangladesh J. Agril. Res. 47(4): 473-476, December 2022 **Short Communication**

FIRST REPORT OF WHITE MOLD CAUSED BY *SCLEROTINIA SCLROTIORUM* ON ZINNIA (*ZINNIA ELEGANS*) IN BANGLADESH

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Zinnia (*zinnia elegans*) is an annual shrub native to Mexico but grown as an ornamental in many places including North America, Africa, Australia Europe and Asia. In Bangladesh, this flower is suitable as beds, pots and borders crops. The cut flowers are commonly used in flower arrangements and decoration. In January 2021, zinnia plants were found infected with white mold symptoms on about 80% plants of the flower beds in front of the guest house of BARI, Gazipur, Bangladesh (23° 59' 20.4504" N, 90° 25' 5.4012" E). The symptoms included wilted, dried and died flower plants (Fig. 1A). Black sclerotia (1.7 to 8.5 × 1.0 to 4.4 mm) were noticed inside the pith cavity of the plant (Fig. 1B). Three flower beds Zinnia (*zinnia elegans*) were surveyed where the incidence was 60% -100 % and higher disease severity was recorded. A good number of Sclerotia, the fruiting bodies of the fungus, were collected from the infected Zinnia plants. The collected sclerotia were surface sterilized with 0.5% NaOCl solution. After washing twice with sterilized distilled water, the sclerotia were plated on PDA (HiMedia, India) medium. After incubating at 25° C for 4 days, identical whitish mycelia were developed (Fig. 1C). Black sclerotia were formed after 15 days of incubation in the dark (Fig. 1D). Harvested sclerotia were irregular in shape. The diameter of sclerotia was 1.8 - 7.2 mm, which was measured based on the average diameter of 25 sclerotia grown on 3 PDA plates. The morphological characters of mycelia and sclerotia were similar to *Sclerotinia sclerotiorum* (Kohn, 1979).

For molecular identification, ITS rDNA gene region was amplified using the primer pair ITS1/ITS4. Mycelia of the isolate (ZMJ) were multiplied on potato dextrose (PD) broth. Mycelial agar plugs were cut aseptically from 3-day-old culture and were transferred to conical flasks containing PD broth. After 3 days of incubation at 26 °C on a rotary shaker at 200 rpm, total genomic DNA was extracted from mycelial mat using Wizard DNA purification kit (Promega Corporation, Madison, WI, USA) following the standard protocol. PCR was performed in a thermal cycler (Bio-Rad PTC-200, California, USA) in total volume of 25 µL PCR reaction containing 12.5 µL GoTaq Green master mix (Promega Corporation, Madison, WI), 9.5 µL nuclease-free water, 1 µL of each primer (10µM), and 1µL template DNA. The PCR condition was: one cycle of denaturation at 95° C for 5 min, followed by 30 cycles at 95° C for 30 s, 55° C for 40 s, 72° C for 1 min, and a final extension at 72° C for 10 min (White *et al*., 1990).

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Fig. 1. A. White mold infected zinnia bed B. Sclerotia inside the pith of plants C. 4 days old culture of *S. sclerotiorum* D. Black sclerotia formation in PDA plate after 15 days of incubation at 25 °C E. Zinnia seedlings showing typical symptoms of white mold infection after artificial inoculation

The amplified PCR product was cleaned using Wizard@SV gel and PCR Cleanup kits (Promega, USA) following the manufacturer's instructions. The purified DNA of the isolate ZMJ was successfully sequenced (National Institute of Biotechnology, Bangladesh) and deposited to GenBank under the accession no. OP102544. The possible identity of the isolate was established by comparing the ITS sequence with those in GenBank database (National Center for Biotechnology Information [NCBI]) under US National Institute of Health, Bethesda, MD, USA. BLAST search analysis showed that the isolate (GenBank OP102545) is 99% identical to the reference strain MT393753, MT378216 and MT177216.

Phylogenetic analysis of the ITS sequence data was done by means of Maximum Composite Likelihood (MCL) method using MEGA 6.0 software. The sequence distance was calculated by Tamura-Nei parameter model (Tamura, 1993). The sequence alignments of the rDNA regions were performed utilizing ClustalW program. Bootstrap values were obtained 1000 replicates to determine the support from each group. In the Phylogenetic tree, the isolate of Bangladesh (OP102545) was placed in distinct *S. sclerotiorum* group with 88% bootstrap support while other species of *Sclerotinia* clustered in different group. Thus, the fungal isolate (ZMJ) was identified as *Sclerotinia sclerotiorum* (Fig. 2).

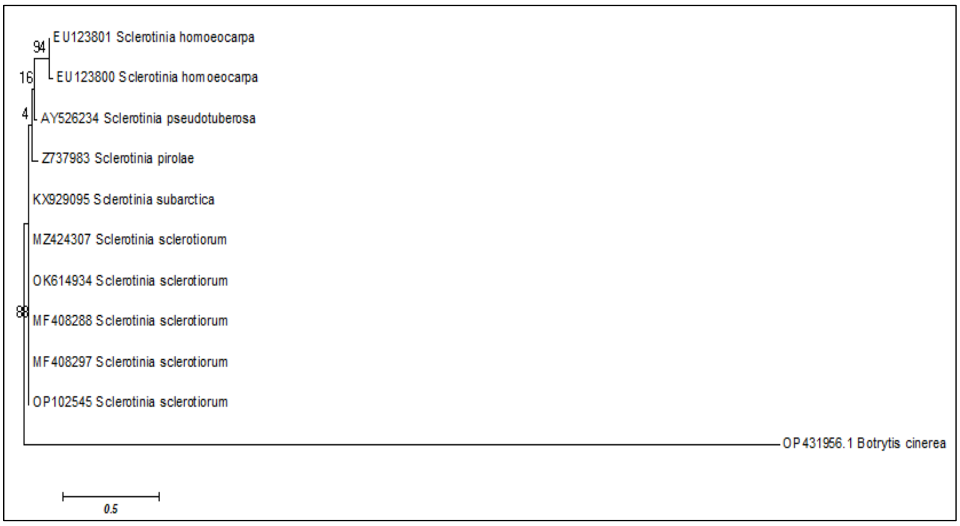

Fig. 2. Phylogenetic tree obtained from ITS region sequences from *Sclerotinia* spp. The numbers above the nodes are the bootstrap values obtained from 1000 replicates. The numbers before taxa are the GenBank accession numbers. Other genus *Botrytis cinerea* was used as an out group

S. sclerotiorum was grown in sterile wheat kernel in 1 Liter conical flasks at 25° C in the Plant Pathology laboratory, BARI, Gazipur. Twenty days old three seedlings of zinnia were grown on one pot (14-cm dia.). Seven days old *S. sclerotiorum* inoculum was incorporated with the sterile surface soil of each pot (3 pots). There were another set of three pots served as control (without inoculum). The experiment was conducted with three replications (three pots inoculated and three pots non-inoculated). The inoculated plants were kept in the pothouse of PPD, BARI at 25° C for 3 weeks. Within 4 days of inoculation, the plants showed wilted symptoms including white necrotic spots on leaves and mycelia covered the stems and surface of soil (Fig. 1E). White cottony mycelia were plated on PDA from the infected stems. The control plants were symptomless and the same deposited isolate (ZMJ) was used for the fulfillment of Koch's postulates. Previously, white mold was recorded in red salvia and marry gold flower in Bangladesh (Islam *et al*., 2019; Rahman *et al*., 2015). To our best knowledge, this is the first report of *S. sclerotiorum* causing white mold disease of *Zinnia elegans* in Bangladesh.

Declaration

Conflict of interest: The authors have declared that they have no conflict of interest.

Research involving human and animal participants: No Human subject or vertebrate animal was used in this study.

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