

First Report of *Rhizoctonia solani* AG 2-IIIB causing Damping-off on Bottle Gourd (*Lagenaria siceraria*) Seedlings in Bangladesh

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Abstract

Bottle gourd is a popular winter vegetable crop in Bangladesh. Bottle gourd seedlings with damping off symptoms were collected in October 2024 from Savar upazila, Dhaka. The causal agent was grown in growth media, and studied the morphological features. Molecular identity was confirmed through amplification of internal-transcribed spacer (ITS) region, RNA polymerase II (*rpb2*), and β -tubulin (*tub2*) genes. Based on morphological and molecular investigations, the pathogen was verified as *Rhizoctonia solani* AG 2–2 IIIB. Pathogenicity test was done to fulfill the Koch's postulates. These findings will be useful to design an effective disease management program of cucurbits in Bangladesh.

Full Text

Bottle gourd (*Lagenaria siceraria* (Mol.) Stand. is originated from Africa, which then widely distributed, and now cultivated in many countries including China, Europe, Haiti, Hawaii, India, Iraq, Turkey, Venezuela, and Bangladesh (Kistler et al. 2014; Richardson 1972). Bottle gourd is an economically important vegetable crop in Bangladesh under Cucurbitaceae family, and a leading source of nutrition in human diet (Rahaman 2003). Bottle gourd is a year round vegetable crop, cultivated in Mymensingh, Manikganj, Rajshahi, Norshingdi, Savar, Jamalpur, Rangpur, and Comilla districts in Bangladesh. Seedlings of bottle gourd can be affected by several diseases such as damping off, wilting, seedling rot etc. in the nursery, greenhouse and in the field conditions (Zitter et al. 1996). In Bangladesh, powdery mildew, virus mosaic disease, gummy stem blight, anthracnose, fruit rot, downy mildew and wilting are the major bottle gourd diseases. Various pathogens including *Colletotrichum*, *Alternaria*, *Pythium*, *Botrytis*, *Cercospora*, *Pseudoperonospora*, *Plectosporium*, *Sphaerotheca*, *Cladosporium*, *Septoria*, *Verticillium* causes significant crop losses in cucurbits worldwide (Sean et al. 2021; Aktaruzzaman et al. 2019).

Rhizoctonia solani is a common seed-and soil-borne necrotrophic fungus which causes various diseases to cultivated fields, ornamental plants, and turf grass. *R. solani* is prevalent in both tropical and temperate region of the world (Salazar et al. 2000). *R. solani* represents remarkable genetic variations and comprises 13-different anastomosis groups (AGs), which are genetically distinctive populations (Ogoshi, 1996; Sharon et al., 2006). This fungus primarily exists as mycelia in nature and also survives for a long time in the soil through resting spore called sclerotia and forms sexual spores every so often (Cubeta and Vilgalys, 1997). Typical damping-off seedling symptom includes a watery rot on the hypocotyl at or near the soil line, light to dark brown lesion. Germinating seed can also be attacked by these fungi before they emerge from the soil (pre-emerging damping off), resulting in poor stands (Paulitz et al. 2006).

In Mid-September 2024, bottle gourd seedlings with dark brown to black discolored were observed from a grower's seed bed. Diseased plants showed damping off symptoms including water soaked lesions in the affected regions with limited feeder roots and rootlets. Affected seedling showed 25 to 30 % disease severity. A severe decline of gourd seedlings were collected and brought to Plant Pathology Laboratory,

Sher-e-Bangla Agricultural University, Dhaka. *Rhizoctonia solani* was consistently isolated from the damping off seedlings of bottle gourd. The aim of this study was to identify and characterize the causal agent of the host disease.

Twenty five bottle gourd seedlings were collected from a grower's commercial nursery located at Savar Upazila under Dhaka District in Mid-October 2024, which had dark brown to black discolored lesions with typical damping-off symptoms (Fig. 1A & 1B). Symptomatic seedlings had approximately 30 to 40% disease severity. Samples were surfaced sterilized and plated on to the potato dextrose agar (PDA) media at 25°C in dark condition for 3 days. Growing hyphal tips were transferred to PDA and clarified V8 (CV8) agar media to observe the pure culture and development of sclerotia. Morphological features were studied under compound microscope and inverted florescent compound microscope at 40X magnification (VWR® ELWD N.A. 0.30). Identification of *R. solani* was done by comparison with morphological characteristics, visual observation, and microscopic study.

R. solani isolates were grown in the PDA media amended with streptomycin sulphate at 200 mg/L for 10 days at room temperature. Genomic DNA was extracted from the pure culture of all isolate using DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol and stored at -20°C for further use. Molecular identification was done by amplification and sequencing of the internal transcribed spacer (ITS, ITS1/ITS4) regions and largest RNA polymerase II subunit (*rpb2*) (O'Donnell et al. 2010), and β -tubulin (*tub2*) (Glass and Donaldson 1995) genes (Table 1). The PCR reaction was carried out in a 25- μ L reaction mixture containing 1.0 μ L of each primer (10 μ mol/ml), 2.0 μ L of genomic DNA (10 ng/ μ L), 12.5 μ L of 2X PCR MasterMix (Promega® GoTaq Green) and 8.5 μ L of nuclease free water. The PCR conditions used were as follows: (i) ITS primers: initial denaturation was performed at 95°C for 10 minutes, followed by 30 cycles in series of 95°C for 1 min, 57°C for 1 min, and 72°C for 90 s, with a final cycle at 72°C for 10 minutes, (ii) *rpb2* primers: 1 cycle of 90 s at 94°C, followed by 40 cycles of 30 s at 94°C, 90 s at 55°C, and 2 min at 68°C, followed final extension for 5 min at 68°C, and for *tub2* primers: 94°C for 1 min; 58 or 68°C for 1 min; 72°C for 1 min; repeat protocol for 32 cycles, with a 5- to 10-s extension time per cycle. The PCR products were run on 1.5% gel stained with SYBER Safe DNA gel stain in 10X TAE buffer to visualize under the UV light. PCR products were cleaned with Exo SAP-IT (Thermo Fisher Scientific) and sent for sequencing at Gencreate Biotech, China.

Bottle gourd seeds were grown into 12 × 10 cm plastic bags containing autoclaved soil at the Sher-e-Bangla Agricultural University, Dhaka-1207 in the greenhouse condition. To fulfill the Koch's postulates, pathogenicity assay of the isolates was conducted on ten bottle gourd (cv. BARI LAU 3) at planting. Seeds were surface sterilized with 70% ethanol before sowing in the plastic bag. The experiment was conducted twice with a completely randomized design (CRD) with four replications and four seeds per replicate each time. Mycelial discs (5 mm) of ten-day old culture of *R. solani* were inoculated to individual seeds at 2.5 cm soil depth for 5 days at 22±2°C with high relative humidity (>90%) in a 14/10-h photoperiod (600-W high-pressure sodium lamps) in the misting chamber and they were kept constantly wet by placing them in trays (40×20×8 cm) filled with 2 to 3 cm of water. The mock inoculation to seeds

was done with agar plug without *R. solani* mycelia. After 5-days, pots were transferred to the greenhouse at 25°C at 14 h photoperiod and evaluated the pathogenicity at 10 days post inoculation.

Five *R. solani* isolates were obtained from the symptomatic bottle gourd seedlings. All isolates were morphologically identical and produced brown to dark brown, fluffy (Fig. 1C & 1D). Hyphae were septate, initially hyaline and become grey to brown at maturity. Hyphae were branched at right angles with a constriction at the base (Fig. 1E). Sclerotia were variable size, dark-brown to tan colored (Fig. 1F), abundant, sparsely distributed to the growth media (Fig. 1C & 1D). The fungus did not produce any conidia and conidiophore. The causal agent was identified as *Rhizoctonia solani* AG 2-2 IIIB (Sneh et al. 1991). The BLASTn of the consensus nucleotide sequences of all isolates for respective loci were identical. Nucleotide sequences of all isolates were submitted to the NCBI GenBank with accessions ITS (PV101462, PV101463), *rpb2* (PV360730, PV360731), and *tub2* (PX548722; PX548723) which were 100% similar to the reference sequences for ITS (FJ492123, KR736348), *rpb2* (OP832150, OP832157), and *tub2* (FJ392720) of *R. solani* AG 2-2 IIIB. Ten day post inoculation, inoculated seeds developed post emergence damping off symptoms which includes brown to black discolored hypocotyl of the emerged seedlings, rot decay, and death of seedlings (Fig. 1A & 1B). Seedlings that emerged from mock inoculated seeds were asymptomatic (Fig. 2C). *R. solani* isolates were consistently re-isolated from the inoculated seedling and morphology of the isolates retrieved from the inoculated plants was identical to the field isolates of *R. solani*.

Rhizoctonia solani is a destructive plant pathogen to agronomic, horticultural, and forest plants which causes significant yield losses annually. In this study, we have investigated and characterized the causal agent of bottle seedling damping off. To our knowledge, this is the first report of *Rhizoctonia solani* AG 2-IIIB causing damping-off on bottle gourd (*Lagenaria siceraria*) seedlings in Bangladesh. *R. solani* considered to be a serious threat to economic production of cucurbits worldwide. Effective management of *R. solani* disease in cucurbits integrates combination of cultural, biological, and chemical methods. Favorable temperature for damping-off in cucurbits and other crops under wet and warm conditions at 20 to 30°C (68 to 86°F) (Minier and Hanson 2021). Damping off seedling results in poor stand establishment, which can occur as a pre-emergence or seed decay (Baker 1970). Fields without a history of *R. solani* diseases should be selected for planting and crop rotation with non-host for at least three years (Buhre et al. 2009). Extra care should be taken if planting done at early to ensure soil temperatures are adequately warm. Poorly drained bed or areas and excessive irrigation to be avoided which increase the disease incidence. Currently, there are no commercial cucurbit varieties with resistant to *R. solani* damping off seedlings in Bangladesh. Therefore, fungicide application is needed for effective disease management of this pathogen. Azoxystrobin, pyraclostrobin (QoIs) and Penthiopyrad, sedaxane, and fluxapyroxad (SDHI) class of fungicides are considered to be most effective in controlling *R. solani* (Balba 2007; Avenot and Michailides 2010; Liu and Khan 2016a; Bartlett et al. 2002). Seed treatment with effective fungicides such as fludioxonil (FRAC 12), Thiram (FRAC M3), tebuconazole (FRAC 3) + trifloxystrobin (FRAC 11), and propiconazole (FRAC 3) are recommended for damping off disease management (Campion et al. 2003; Sharma et al. 2021). Foliar or in-furrow application of Azoxystrobin and pyraclostrobin (FRAC 11) is very effective for management of *R. solani*. However, repeated use of

single site mode of action of fungicides leads the development of resistance in pathogens populations. It has been reported that the QoI and SDHI fungicides have high and medium to high risk of developing resistance (FRAC 2024). Biological control of *R. solani* spp. with antagonistic bacteria, *Bacillus* spp. *Burkholderia* and *Pseudomonas* sp. and some fungal species such as *Trichoderma harzianum*, and *T. hamatum* have shown excellent efficacy (Verdoodt, 2023; Anitha and Das 2011; Aydin 2022; Farhaoui et al. 2023). This pathogen has wide host ranges and distributed in both the temperate and tropical climatic conditions and capable causing disease in many economically important crops including cereals, vegetables, ornamental and horticultural crops (Salazar et al. 2000). *R. solani* AG 2-2 has been reported to be destructive to many crops includes dry bean, soybeans, corn etc. (Sneh et al. 1991; Englkes and Windels 1996; Harveson 2009).

In the recent past, it has been reported that the bottle gourd seedlings at emergence has increased in location including Manikganj, Tangail, Netrokona, Jamalpur, Rangpur, Niphamari, Savar, and Norshingdi areas. Identification of the seedling damping off disease in cucurbits was confirmed only based on host symptoms. We have studied the morphological features of the *Rhizoctonia solani* AG 2-2IIIB followed by microscopy, pathogenicity and molecular characterization through multilocus sequence typing. To our knowledge, this is the first report of *R. solani* AG-2-2 IIIB causes seedling damping off bottle gourd in Bangladesh. This finding will be useful to design an effective disease management program to ensure economic production of cucurbits in Bangladesh.

Declarations

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Author contributions

Dr. Md Ziaur Rahman Bhuiyan: Conceptualization, investigation, supervision, resources and writing – original draft. **Mahmuda Akter:** Writing – review & editing. **Dr. Nazneen Sultana:** Writing–review & editing. **Md. Jahidul Islam:** Writing – review & editing. **Md. Akash Miah:** Isolation, microscopy, and identification of the pathogen, and **Md. Ferdous Alom:** Isolation, microscopy, and identification of the pathogen.

Conflict of interest

The authors declare that they have no conflict of interest.

Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors.

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Table

Table 1. Primers were used in this study.

Locus	Primer	Primer Sequence (5'-3')	Reference
ITS	ITS1	5'-TCCGTAGGTGAACCTGCGG-3'	White et al. 1990
	ITS4	5'-TCCTCCGCTTATTGATATGC-3'	
<i>rpb2</i>	5F2	5'- GGGGWGAYCAGAAGAAGGC-3'	O'Donnell et al. 2010
	7cR	5'- CCCATRGCTTGYTTRCCCAT-3'	
<i>tub2</i>	Bt2a	5'-GGTAACCAAATCGGTGCTGCTTTC-3'	Glass and Donaldson 1995
	Bt2b	5'-ACCCTCAGT GTAGTG ACC CTTGGC-3'	

Figures

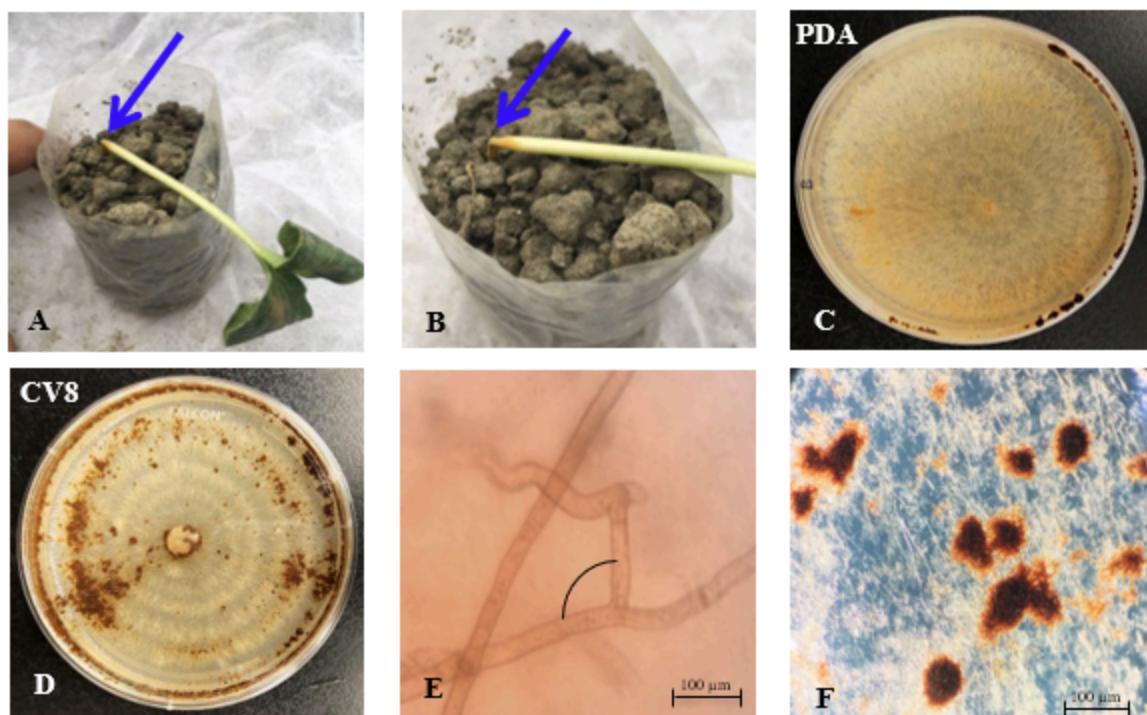


Figure 1

(A-B) Bottle gourd seedlings had dark brown to black water soaked lesions (blue arrows) were collected from the nursery, (C-D) pure colonies of *R. solani* grown in the PDA and CV8 media had brown to blackish mycelial mass and abundant sclerotia, (E) septate mycelia, branched at right angles with a constriction at the base showing 90° angle to parental hyphae (scale bar=100μm), (F) sclerotia were dark brown to tan colored (scale bar=100μm).

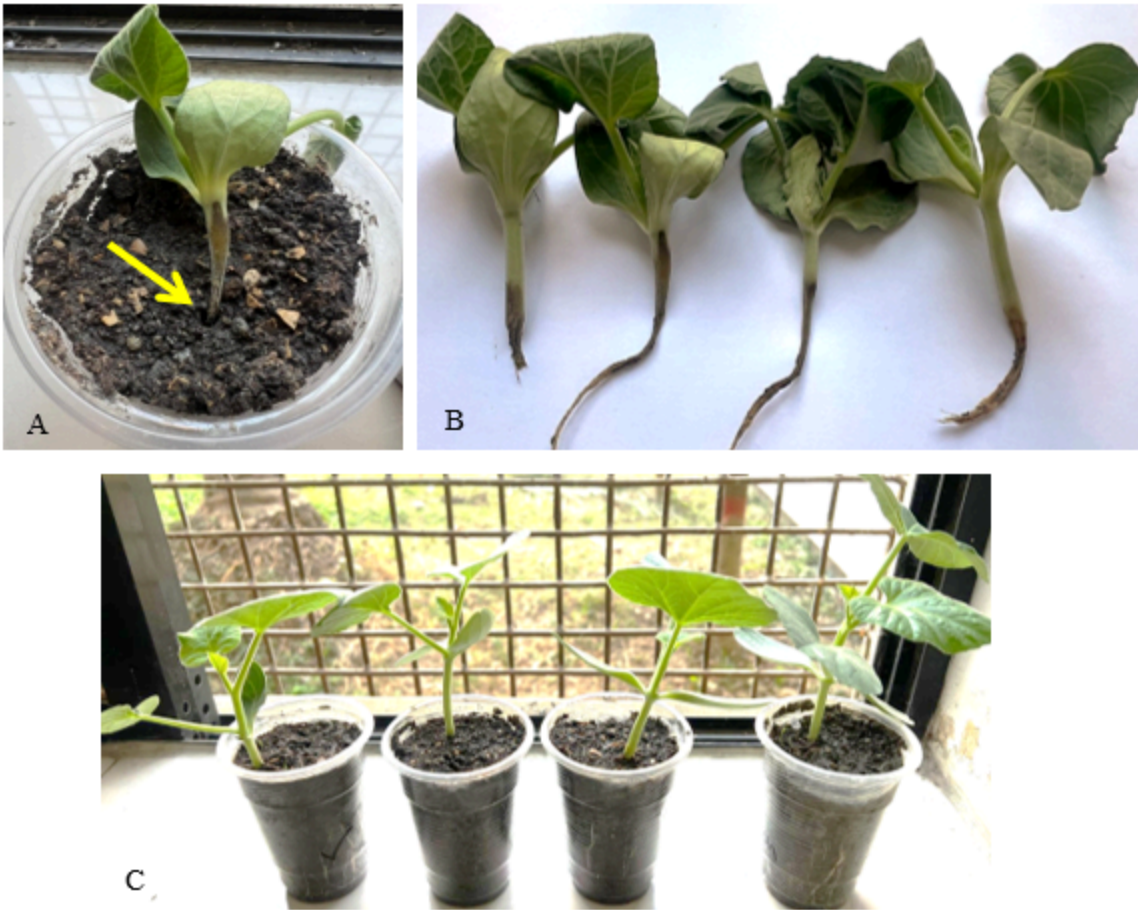


Figure 2

Pathogenicity assay; (A-B) *R. solani* AG 2-2IIIB inoculated seedlings developed brown to black lesion on the hypocotyl, and damping off symptoms on the bottle gourd seedlings, while (C) the mock inoculated seedlings were symptomless.