

Case Report

First report of *Alternaria alternata* causing leaf spot on cabbage (*Brassica oleracea* var. *capitata*) in Bangladesh

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Abstract: Cabbage (*Brassica oleracea* var. *capitata*) is one of the major winter vegetables grown in Bangladesh, recognized for its dietary value and economic importance. During November 2023, cabbage leaves exhibiting typical leaf spot symptoms were collected from the Bangladesh Agricultural Research Institute (BARI), Gazipur, to identify the associated pathogen. The infected leaves showed dark brown to black necrotic lesions with concentric rings characteristic of *Alternaria* leaf spot. The pathogen was isolated on Potato Dextrose Agar (PDA) and purified using a single-conidium isolation technique. Morphological characterization revealed colony and conidial features consistent with *Alternaria alternata*. To confirm the pathogen identity, molecular characterization was performed using the internal transcribed spacer (ITS) region of rDNA. Amplification with ITS1/ITS4 primers generated a 510 bp fragment, and the resulting sequence was deposited in GenBank under accession number OP585635. Comparison of the obtained sequence against the GenBank database revealed a high degree of homology (99%) with previously deposited *A. alternata* sequences. The phylogenetic tree also placed the isolate in the same evolutionary group as *A. alternata*, with 88% bootstrap support. Species-specific PCR using *A. alternata*-specific primers produced the expected 184 bp amplicon, providing additional confirmation of species identity. Pathogenicity was evaluated using a detached-leaf assay. Leaves inoculated with the fungal isolate exhibited characteristic disease symptoms comparable to those recorded in naturally infected plants, while no symptoms were detected on the control leaves. Following symptom development, the fungus was recovered from infected tissues and its identity was verified using an *A. alternata*-specific PCR assay, satisfying the requirements of Koch's postulates. The combined evidence obtained from cultural and microscopic observations, ITS-rDNA sequencing, phylogenetic inference, species-specific molecular detection, and pathogenicity assessment demonstrated that *A. alternata* was responsible for the leaf spot symptoms observed on cabbage. To our knowledge, this study constitutes the first molecularly confirmed record of *A. alternata* infecting cabbage in Bangladesh. The information generated will support future disease monitoring efforts and contribute to the formulation of appropriate management practices for sustainable cabbage production.

Keywords: Brassicaceae; concentric ring; ITS1/ITS4; species-specific PCR; detached leaf

1. Introduction

Belonging to the family Brassicaceae, cabbage (*Brassica oleracea*) is among the most widely grown vegetable crops and contributes significantly to global vegetable production. Several biotic and abiotic factors constrain the growth, productivity, and quality of cabbage and other cruciferous crops. Among these, pre-harvest and post-harvest losses caused by plant pathogens are particularly important, as they significantly reduce crop yield and market value (Oloyede *et al.*, 2021). Therefore, the development and implementation of disease-free crop production practices are essential for sustainable cabbage cultivation.

Alternaria leaf spot poses a major constraint to cabbage cultivation in many regions of the world. Several *Alternaria* species have been implicated in the disease, and their host range extends beyond Brassicaceae to include economically important plants belonging to the Solanaceae and Cucurbitaceae families. Symptoms typically appear as small dark specks that gradually enlarge into circular to irregular brown or black lesions with concentric rings and chlorotic halos (Kumar *et al.*, 2024).

Alternaria spp. are familiar as major pathogens of cabbage and may cause yield losses of up to 60%, depending on environmental conditions and host susceptibility (Bhushan *et al.*, 2025). Among the *Alternaria* species affecting cruciferous vegetables, *A. brassicicola*, *A. brassicae*, and *A. japonica* have long been recognized as the major pathogens responsible for leaf spot development (Kushwaha and Arsia, 2024). These pathogens have been documented in broccoli, cauliflower, cabbage, and Brussels sprouts. For example, *A. brassicicola* as the sole causal agent of cabbage leaf spot in Karnataka, India (Sohil *et al.*, 2025). Similarly, *A. brassicae* caused leaf spot in Chinese cabbage, whereas *A. brassicicola* was associated with disease development in cabbage, cauliflower, and broccoli (Michereff *et al.*, 2012).

In addition to causing foliar diseases, *Alternaria* species can infect seeds, seedlings, and pods, thereby affecting crop establishment and productivity (Köhl *et al.*, 2010). Al-lami *et al.* (2020) identified four major pathogenic species affecting cruciferous crops, namely *A. alternata*, *A. brassicae*, *A. brassicicola*, and *A. japonica* (syn. *A. raphani*). All four species are seed-borne and have been detected in different parts of infected seeds, facilitating pathogen dissemination and disease transmission (Saharan *et al.*, 2016).

Beyond their agricultural significance, some *Alternaria* species have emerged as important human health concerns. Airborne spores of *Alternaria* are known allergens and have been associated with respiratory disorders, particularly asthma. The major allergen *Alt a1* is considered a marker of primary sensitization to *Alternaria* spp. (Hernandez-Ramirez *et al.*, 2021). Therefore, accurate identification of *Alternaria* species is important not only for disease management but also for understanding their broader environmental and public health implications (Rahimloo and Ghosta, 2015).

Species identification based solely on morphological features can be challenging in *Alternaria*, as numerous species exhibit overlapping colony traits and microscopic structures. Consequently, molecular tools such as polymerase chain reaction (PCR) and DNA sequencing have become indispensable for reliable identification. Several genetic markers, including the internal transcribed spacer (ITS) region, actin, calmodulin, *Alt a1*, chitin synthase, histone H3, and β -tubulin genes, have been widely employed in taxonomic and phylogenetic studies of *Alternaria* species (Elfar *et al.*, 2018; Kiasat *et al.*, 2022).

Despite the economic importance of cabbage cultivation in Bangladesh, information regarding the species composition of *Alternaria* associated with cabbage leaf spot remains scarce. Although *Alternaria* diseases have been reported on several cruciferous crops, there is limited molecular evidence identifying the specific *Alternaria* species responsible for cabbage leaf spot in Bangladesh. This lack of information hampers accurate disease diagnosis, epidemiological investigations, and the development of effective management strategies. Therefore, a clear understanding of the causal pathogen is urgently needed to support disease surveillance and sustainable cabbage production.

Considering the symptom expression and the established association of *Alternaria* species with leaf spot diseases of Brassica crops, it was hypothesized that an *Alternaria* species was responsible for the disease observed in cabbage. To test this hypothesis, the pathogen was examined through an integrated approach involving morphological, molecular, and phylogenetic methods. The central research question addressed in this study was, which species of *Alternaria* is associated with leaf spot disease of cabbage in Bangladesh, and can its identity be confirmed through morphological characterization, molecular detection, phylogenetic analysis, and pathogenicity testing? Accordingly, the objective of the present study was to identify the *Alternaria* species associated with cabbage leaf spot disease using morphological characterization, ITS-rDNA sequencing, species-specific PCR, phylogenetic analysis, and pathogenicity assays.

The novelty of this study lies in the integrated application of morphological, molecular, phylogenetic, and pathogenicity-based approaches to identify the causal agent of cabbage leaf spot in Bangladesh. To the best of our knowledge, no previous study has provided molecular confirmation of *A. alternata* associated with cabbage

leaf spot in the country. Thus, this work represents the first comprehensive report of this pathogen-host association in Bangladesh. The findings of this study will contribute to the accurate diagnosis and monitoring of cabbage leaf spot disease and provide baseline information for future epidemiological investigations. The outcomes of this study are expected to provide valuable information for the development of resistant germplasm, improvement of seed certification procedures, and design of comprehensive disease control measures for sustainable cabbage cultivation.

2. Materials and Methods

2.1. Ethical approval

Ethical approval was not required for the study.

2.2. Study area and sample collection

Symptomatic cabbage leaves of the cultivar BARI Badhakapi-2 were sampled in December 2023 from the experimental field of the Plant Pathology Division, BARI, Gazipur, Bangladesh (Figure 1). The collected specimens were placed in paper bags, delivered to the laboratory, and immediately prepared for fungal isolation and subsequent analyses.

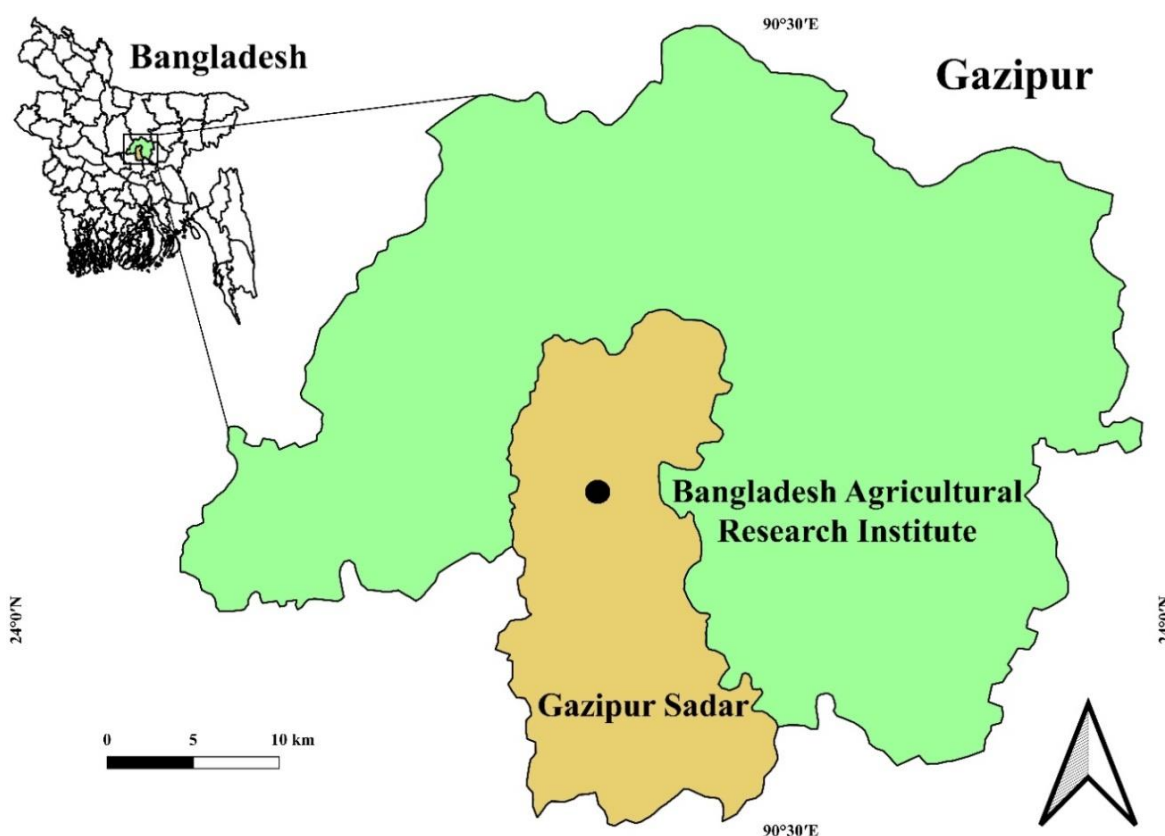


Figure 1. The symptomatic infected leaves were collected from BARI, Bangladesh.

2.3. Isolation and morphological characterization of the pathogen

Leaf samples displaying typical disease symptoms were first cleaned under flowing tap water to remove surface contaminants. The tissues were then disinfected by immersion in 0.5% sodium hypochlorite solution, followed by three successive rinses with sterile distilled water to remove any remaining sterilizing agent. After drying on sterile filter paper, small pieces were excised from the interface between healthy and infected tissue and transferred onto Potato Dextrose Agar (PDA). The cultures were maintained at 25 °C for seven days to allow fungal development. To obtain pure cultures, conidia were harvested from colonies that had been sporulating for 7–14 days. The spores were dispersed on 2% water agar plates using a sterile loop and incubated at 22 °C for approximately 18–20 h to facilitate germination. Individual germinated spores were identified under a dissecting microscope and carefully transferred to fresh PDA plates. The resulting single-spore cultures were subsequently used for examination of colony morphology and conidial characteristics to support the preliminary identification of the fungal pathogen.

2.4. Molecular identification

Fungal biomass produced in Potato Dextrose Broth (PDB) was used for DNA extraction with the Wizard® Genomic DNA Purification Kit (Promega Corporation, Madison, WI, USA), following the supplier's recommended procedure. Species identification was initially assessed by amplifying the internal transcribed spacer (ITS) region of ribosomal DNA using the universal primers ITS1 and ITS4 (Nilsson *et al.*, 2019). Amplification products were separated on 2% agarose gels containing 1× TAE buffer and subsequently examined under UV illumination. The target PCR fragments were purified using the Wizard® SV Gel and PCR Clean-Up System (Promega, USA) prior to sequencing. DNA sequencing was carried out by the National Institute of Biotechnology (NIB), Bangladesh. The resulting sequences were edited and aligned with reference sequences using the ClustalW alignment tool to facilitate comparative analysis and taxonomic assessment. To provide additional confirmation of the pathogen identity, a species-specific PCR assay was performed using the primer set Aalt F (5'-GTGCCTTCCCCCAAGGTCTCCG-3') and Aalt R (5'-CGGAAACGAGGTGGTTCAGGTC-3'), which specifically targets *A. alternata* (Achilonu *et al.*, 2023). Detection of the expected amplification product served as supplementary molecular evidence for confirming the isolate at the species level.

2.5. Pathogenicity test

A detached-leaf bioassay was performed to determine whether the recovered fungal isolate could induce disease symptoms on cabbage. Cultures grown on PDA for one week at 25 °C under a 12:12 h light–dark regime were used as the inoculum source. Leaves obtained from healthy 40-day-old cabbage plants (cv. BARI Badhakapi-2) were surface-disinfected and thoroughly rinsed with sterile water before inoculation. Small artificial wounds were introduced into the leaf tissue, after which mycelium-bearing agar plugs were applied to the inoculation sites. Control leaves received identical agar plugs lacking fungal growth. All leaves were maintained in humid chambers prepared with sterile Petri dishes and moistened filter paper and incubated for two weeks at 25 °C. Humidity was maintained throughout the assay by periodic addition of sterile water. Symptom expression was monitored throughout the incubation period. The experiment was arranged with three replicates per treatment and repeated independently. Following symptom development, the pathogen was re-isolated and molecularly verified using species-specific primers targeting *A. alternata*. Recovery of the same organism from inoculated tissues provided evidence supporting Koch's postulates.

2.6. Statistical analysis

Phylogenetic analysis was conducted using MEGA version 11.0 based on the Maximum Likelihood approach, with evolutionary distances estimated using the Tamura–Nei model (Tamura *et al.*, 2021). Bootstrap support values were calculated from 1,000 resampled datasets to determine the confidence of the phylogenetic groupings.

3. Results and Discussion

3.1. Symptoms of *Alternaria* leaf spot and morphological characteristics of the pathogen

An estimated 37% of the cabbage crop surveyed showed signs of leaf spot infection, and symptomatic leaves were selected for laboratory analysis%. Initial symptoms appeared on older leaves as small, dark specks that gradually enlarged into circular to irregular necrotic lesions. As the disease progressed, the lesions became dark brown to black with distinct concentric rings and slightly sunken centers. In advanced stages of infection, adjacent lesions coalesced, resulting in extensive blighting and eventual death of the affected leaf tissue. The symptoms observed in the present study were consistent with those previously reported for *Alternaria* leaf spot of cabbage and other cruciferous crops (Valvi *et al.*, 2019). Following incubation on PDA at 25 °C for 7 days, the fungal isolate produced slow-growing colonies with a raised, fluffy appearance and distinct white margins. Colony pigmentation varied from greenish to olive-brown, while young aerial hyphae appeared whitish, particularly in the central region of the colony (Figure 2A). Microscopic examination revealed brown-colored conidia that were muriform, obclavate to ellipsoidal, smooth-walled, and borne singly or in short chains. The conidia possessed 3–5 transverse septa and a short beak. Conidial dimensions ranged from 19.0 to 42.8 µm in length and 6.5 to 18.2 µm in width (Figure 2B).

The isolate produced brown, septate conidiophores that were either simple or sparsely branched, a feature consistent with the taxonomic descriptions of *A. alternata* (Kidd *et al.*, 2023). Isolates displaying typical *Alternaria* morphology, characterized by pigmented mycelia and multicellular septate conidia, were purified through single-spore culture to ensure genetic uniformity (Bessadat *et al.*, 2021). The observed colony appearance and growth characteristics closely resembled those previously documented for *A. alternata* (Patel *et*

al., 2023). To maintain the isolate for future studies, mycelial cultures were preserved in 50% glycerol at -80°C . For future reference and study, the fungal isolate was archived in the culture bank of the Plant Pathology Division, BARI, Gazipur, Bangladesh, where it was registered as BAPCC 120.

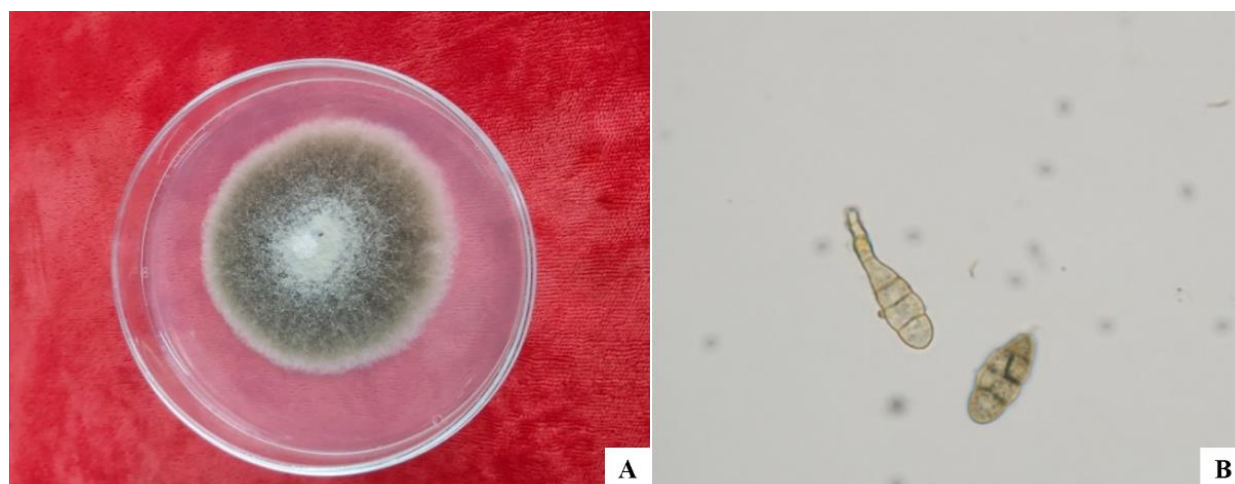


Figure 2. A. Colony of *Alternaria alternata* on PDA plate after 7 days of inoculation; B. Muriform conidia of *A. alternata*.

3.2. Molecular identification

For species determination, a DNA-based approach targeting the ITS region of rDNA was employed. The sequence obtained from the isolate was deposited in the GenBank database and made available under accession number OP585635. A BLAST search against the NCBI GenBank database revealed that the obtained sequence shared 99% nucleotide identity with previously reported *A. alternata* isolates. Phylogenetic reconstruction further demonstrated that the Bangladeshi isolate from cabbage clustered within the *A. alternata* lineage and grouped closely with reference isolates of the same species. The isolate formed a strongly supported clade with a bootstrap value of 88%, providing robust evidence for its taxonomic placement as *A. alternata* (Figure 3). The molecular identification obtained in this study is consistent with previous reports that highlighted the usefulness of ITS sequence analysis for the accurate discrimination and confirmation of *Alternaria* species (Dettman and Eggertson, 2021).

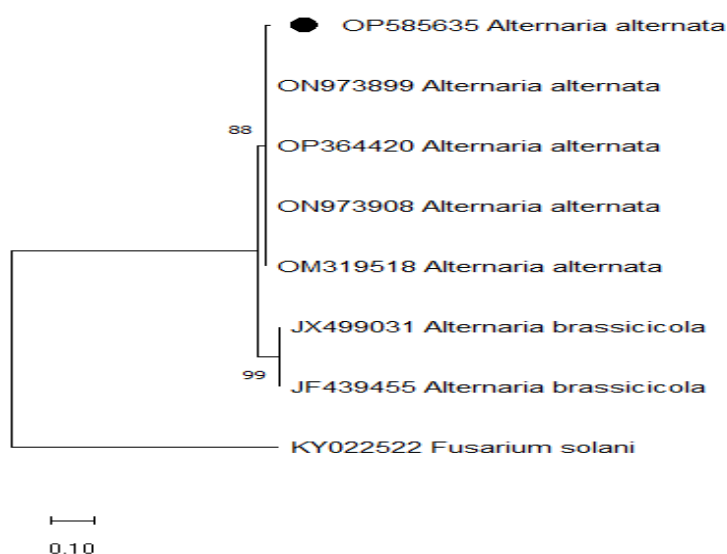


Figure 3. Phylogenetic relationships among *Alternaria* species inferred from ITS-rDNA sequence data. Values displayed at the nodes represent bootstrap support percentages calculated from 1,000 replicates. GenBank accession numbers are provided before each taxon name. The isolate characterized in the present study (*A. alternata*, OP585635) is highlighted in bold.

Species-specific PCR using the primer pair Aalt F and Aalt R generated the expected amplicon of 184 bp (Figure 4). The presence of this diagnostic fragment provided additional evidence for the identification of the isolate as *A. alternata*. The amplicon size obtained in the present study was consistent with previous reports and further validated the species-level identification of the pathogen (Kordalewska *et al.*, 2015).

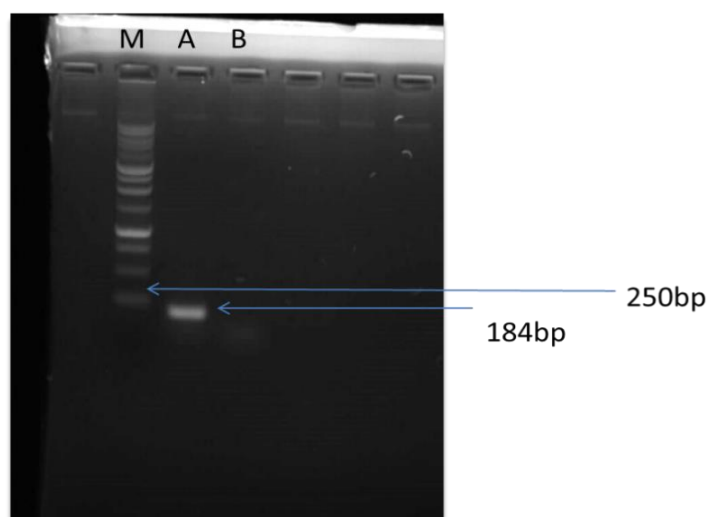


Figure 4. Agarose gel electrophoresis of DNA of *Alternaria alternata* (184 bp) ran on 2% agarose gel when stained with cyber green. Lane M: 100 bp DNA ladder, Lane A: *A. Alternata* isolate OP585635 and Lane B: negative control.

3.3. Pathogenicity test

The pathogenicity of the isolate was evaluated using a detached-leaf assay on young cabbage leaves. Typical disease symptoms developed within 2 days after inoculation and closely resembled those observed under natural field conditions. Inoculated leaves exhibited characteristic necrotic lesions with concentric rings, whereas no symptoms were observed on the control leaves treated with sterile PDA plugs (Figure 5). The pathogen was recovered from symptomatic lesions and further examined by species-targeted PCR to establish its involvement in symptom expression. The molecular profile of the re-isolated fungus confirmed its identity as *A. alternata*, thereby fulfilling Koch's postulates. Similar findings have been reported by Ertay (2022), who successfully confirmed the pathogenicity of *A. alternata* through re-isolation and molecular verification. The symptoms induced by the isolate were comparable to those described in previous studies on *Alternaria* leaf spot of cruciferous crops (Patel *et al.*, 2023; Ohene-Mensah *et al.*, 2025). Furthermore, *A. alternata* has been demonstrated to be pathogenic on Chinese cabbage, highlighting its ability to infect a broad range of Brassica hosts (Shi *et al.*, 2021). In Bangladesh, the pathogen has previously been reported on broccoli leaves (Nira *et al.*, 2022).

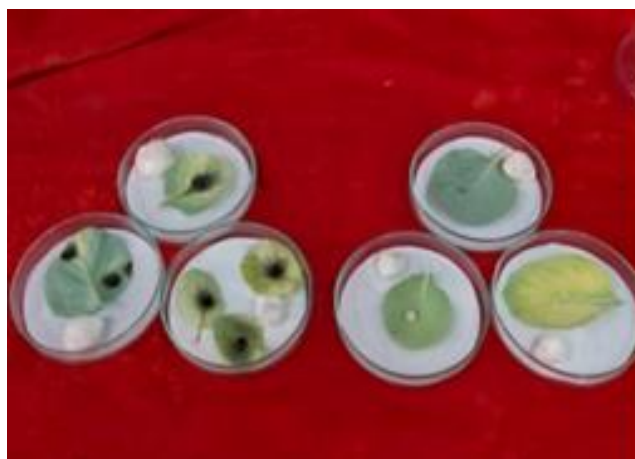


Figure 5. Pathogenicity test on detached cabbage leaves.

4. Conclusions

The present study conclusively identified *A. alternata* as the causal agent of cabbage leaf spot disease in Bangladesh through an integrated approach involving morphological characterization, molecular detection, phylogenetic analysis, and pathogenicity testing. The isolate exhibited morphological features typical of *A. alternata*, while ITS-rDNA sequencing, species-specific PCR, and phylogenetic analysis provided robust molecular evidence supporting its identification. Pathogenicity assays further confirmed its disease-causing ability and fulfilled Koch's postulates. To our knowledge, the occurrence of leaf spot caused by *A. alternata* on cabbage has not previously been reported from Bangladesh, and the current study therefore constitutes the first authenticated report. This finding expands the current knowledge of *Alternaria*-associated diseases affecting cruciferous crops in the country and provides valuable baseline information for disease diagnosis and surveillance.

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Data availability

The ITS gene of the pathogen has been deposited in the NCBI GenBank database (OP585635).

Conflict of interest

None to declare.

Authors' contribution

Ferdous-E-Elahi: material preparation, data collection, analysis, original draft; Md. Mahamudul Hasan, Monirul Islam and Md. Abu Raihan: conceptualization, investigation, editing; Md. Monirul Islam, Tasfia Hassan Tabassum and Sayma Tahsin Nira: re-writing, review. All authors have read and approved the final manuscript.

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