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## SEED HEALTH ASSESSMENT AND MOLECULAR CHARACTERIZATION OF *Alternaria* Spp. IN SUSCEPTIBLE SESAME GENOTYPES

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### ABSTRACT

Sesame (*Sesamum indicum* L.) is a major oilseed crop affected by seed-borne fungal pathogens that reduce seed quality and productivity. Among them, *Alternaria alternata* is one of the most destructive, causing leaf blight and seed infection. The present study was conducted at

S.V. Agricultural College, Tirupati (2025), to assess the seed health status of ten susceptible sesame genotypes using the standard blotter and agar plate methods. The blotter method revealed six seed-borne fungi, with *Alternaria* spp., showing the highest incidence (63.58%), followed by *Aspergillus flavus* (29.78%), while *Helminthosporium* spp., *Curvularia* spp., *A. niger* and *Rhizopus* were recorded in lower frequencies. The agar plate method detected seven fungi, with *Alternaria* spp., again predominant (32.87%), followed by *A. niger* (28.61%) and *Rhizopus* spp. (19.34%). *A. flavus*, *Helminthosporium* spp., *Endophoma* spp. and *Curvularia* spp., occurred at lower incidences. Pure cultures of *Alternaria* spp. were isolated and morphological characterisation confirmed the pathogen as belonging to the *Alternaria* genus. Molecular identification using ITS1 and ITS4 primers amplified a 550 bp fragment, which showed 100% sequence similarity with *A. alternata* reference isolates in NCBI. Phylogenetic analysis clustered the test isolate with known *A. alternata* strains, confirming its identity. The study highlights the predominance of *A. alternata* in sesame seeds and the importance of integrating morphological and molecular approaches for accurate pathogen detection. These findings provide a basis for effective seed health management and strategies to reduce inoculum levels in sesame cultivation.

**Key words:** Seed health testing, Seed-borne fungi, *Alternaria alternata*, Seed quality, Molecular identification

### Introduction

Sesame (*Sesamum indicum* L.) is an important oilseed crop valued for its high-quality edible oil, nutritional benefits and medicinal properties. Despite its economic significance, sesame cultivation is constrained by several biotic stresses, of which seed-borne fungal pathogens pose a major threat to seed quality, germination and crop productivity. Among these, *Alternaria* spp. is one of the most destructive pathogens, responsible for leaf blight and seed infection, leading to significant yield losses. Other fungi such as *Aspergillus flavus*, *A. niger*, *Curvularia* spp., *Helminthosporium* spp., *Rhizopus* spp. and *Endophoma* spp. are also associated with sesame seeds,

either as field or storage fungi, further reducing seed viability and quality.

Seed-borne pathogens not only affect seed germination but also act as primary sources of inoculum for disease outbreaks in the field, making seed health testing a crucial step in crop protection and quality assurance. Techniques such as the standard blotter method and agar plate method are widely employed to detect fungal contaminants due to their simplicity, reliability and ability to support fungal growth and sporulation. These methods allow the detection of both externally and internally seed-borne fungi, providing insight into pathogen diversity and infection levels across different genotypes.

Morphological identification of *A. alternata* is often challenging due to its close resemblance to other small-spored *Alternaria* species. Furthermore, species within the *Alternaria* section *Alternaria* exhibit high genetic similarity, complicating accurate differentiation. To address this, molecular techniques have been employed, with primers targeting the ITS region, histone 3 and RPB2 genes proving effective in distinguishing *A. alternata* from related taxa. Sequencing of these amplified regions allows precise confirmation of the pathogen's identity and its phylogenetic placement.

The present study was undertaken to evaluate seed health status in highly susceptible sesame genotypes using standard blotter and agar plate methods, to isolate and characterise *Alternaria* spp. and to confirm its identity through morphological and molecular tools. These findings are expected to enhance understanding of the seed-borne nature of pathogens in sesame and to aid in developing effective seed health management and integrated disease management strategies.

### Materials and Methods

This study was carried out in the laboratories of Seed Science and Technology and Plant Pathology, S.V. Agricultural College, Tirupati during 2025. In this study ten highly susceptible sesame genotypes were selected from the field screening based on disease rating scale (0-9) (Mayee and Datar, 1986) and PDI for *Alternaria* spp. infection were selected for seed health assessment. Ten susceptible genotypes include SI-253-1-C, PT-10, SI-2174-1, GKVKS-112, IS-208, RT-392, NIC-10621-13, IS-475, JCS-DT-26, IS-4 for which, seed health tests viz., standard blotter method and agar plate method were conducted.

#### Standard blotter method

Hundred seeds from each of ten susceptible sesame genotypes (IS-4, RT-392, SI-253-1-C, IS-208, PT-10, SI-2174-1, NIC-10621-13, GKVKS-112, IS-475, JCS-DT-26) were tested using the standard blotter method in four replications. Three layers of moistened blotter papers (90 mm) were placed in sterilized petri plates, with 25 seeds arranged in a pattern (16 in an outer ring, 8 in an inner ring and 1 in the center). The plates were incubated at 25±2°C under alternating 12-hour light/dark cycles for 8 days. Distilled water was added every fourth day to maintain moisture. After 8 days, seeds were examined under a compound microscope for fungal pathogens.

#### Agar plate method

Hundred seeds from each of ten susceptible sesame genotypes (IS-4, RT-392, SI-253-1-C, IS-208, PT-10, SI-

2174-1, NIC-10621-13, GKVKS-112, IS-475, JCS-DT-26) were tested using the agar plate method in four replications. Twenty-five seeds were placed per petri plate containing 20 ml of potato dextrose agar. The plates were incubated as per the standard blotter method. After eight days, fungal growth on the seeds was examined under a compound microscope.

#### Per cent seed Infection

The incidence of fungus was recorded as suggested by Aslam *et al.*, (2015) by counting the number of seed colonized by fungus in each replication and the per cent colonization of seed was calculated as follows.

$$\text{Per cent Seed Infection} = \frac{\text{Number of infected seeds}}{\text{Total number of seeds}} \times 100$$

#### Isolation and Identification of *Alternaria* spp.

*Alternaria* spp. was isolated from infected sesame seeds by placing them on potato dextrose agar (PDA) under aseptic conditions in a laminar airflow chamber and incubating at 25±2°C for seven days. The initial fungal growth was sub-cultured onto PDA slants. Purification was done using single spore isolation by preparing a spore suspension from 10-day-old cultures. The suspension was diluted to 10 spores/ml and poured onto 2% sterilized water agar. After 2 hours of incubation, well-separated spores were identified under a compound microscope, marked and aseptically transferred along with a small agar block onto PDA slants using a sterilized scalpel. The culture was maintained by sub-culturing at 8–10 days intervals for further studies. Identification of the fungus was based on colony morphology, hyphae, conidiophores and conidia, with measurements compared to standard descriptions provided by the Common wealth Mycological Institute (CMI, 1952).

#### Morphological and Molecular Characterization of *Alternaria* spp.

The morphological features of the test pathogen were studied from 10-day-old pure cultures grown on PDA and examined under a fluorescence microscope at 40X magnification. Observations included conidial length, width, colour and shape, along with the number of transverse and longitudinal septa per conidium, which provided initial evidence for identification of *Alternaria* spp. For molecular characterization, fungal isolates were purified by the single-spore method and cultured on potato dextrose agar medium. Identification was carried out using both morphological characters and ITS rRNA sequencing, which enabled accurate detection of species identity and the obtained sequences were later submitted to NCBI for reference.

Genomic DNA was extracted from mycelial mats harvested from potato dextrose broth cultures using the CTAB method (Doyle and Doyle, 1990). Approximately 0.5 g of mycelium was ground in liquid nitrogen and suspended in CTAB extraction buffer at 65°C for 45 minutes. Phenol:Chloroform:Isoamyl alcohol (25:24:1) was added, centrifuged and the aqueous phase was purified with chloroform:isoamyl alcohol (24:1). DNA was precipitated with ice-cold isopropanol, pelleted by centrifugation, washed twice with 70% ethanol, air-dried and dissolved in TE buffer. RNA contamination was removed with RNase treatment at 37°C for one hour. The extracted DNA was resolved on 0.8% agarose gel, quantified using a Nanodrop spectrophotometer and the purity checked by A260/A280 nm ratio before storage at -20°C.

PCR amplification was performed with universal fungal primers ITS1 and ITS4 in a 50 µl reaction mixture containing template DNA, PCR buffer, MgCl<sub>2</sub>, dNTPs, primers and Taq DNA polymerase. The amplification program consisted of an initial denaturation at 94°C for 5 minutes, followed by 40 cycles of denaturation at 94°C for 30 seconds, annealing at 50°C for 30 seconds, extension at 72°C for one minute and a final extension at 72°C for seven minutes. PCR products were resolved on 1.2% agarose gel stained with ethidium bromide and visualized alongside a 1 kb DNA ladder. The amplified fragments were purified and sequenced at Barcode Biosciences, Bangalore, using both ITS1 and ITS4 primers.

The sequences were deposited in the NCBI database and subjected to homology search using BLASTn analysis. Closely related sequences were aligned and phylogenetic analysis was carried out with MEGA 11 software to confirm the taxonomic position of the isolates. Molecular data provided precise confirmation of the morphological identification of *Alternaria* spp. Thus, the combined approach of morphology and molecular tools ensured reliable identification. Morphological studies gave initial evidence, while molecular sequencing validated and confirmed the species identity. This integrated characterization of *Alternaria* isolates not only established their identity but also laid a strong foundation for pathogenicity studies and the development of effective management strategies.

## Results and Discussion

The standard blotter method revealed six seed-borne fungi from ten susceptible sesame genotypes (Plate 1). Among them, *Alternaria* spp. showed the highest incidence (63.58%), followed by *Aspergillus flavus* (29.78%), while *Helminthosporium* spp., *Curvularia*

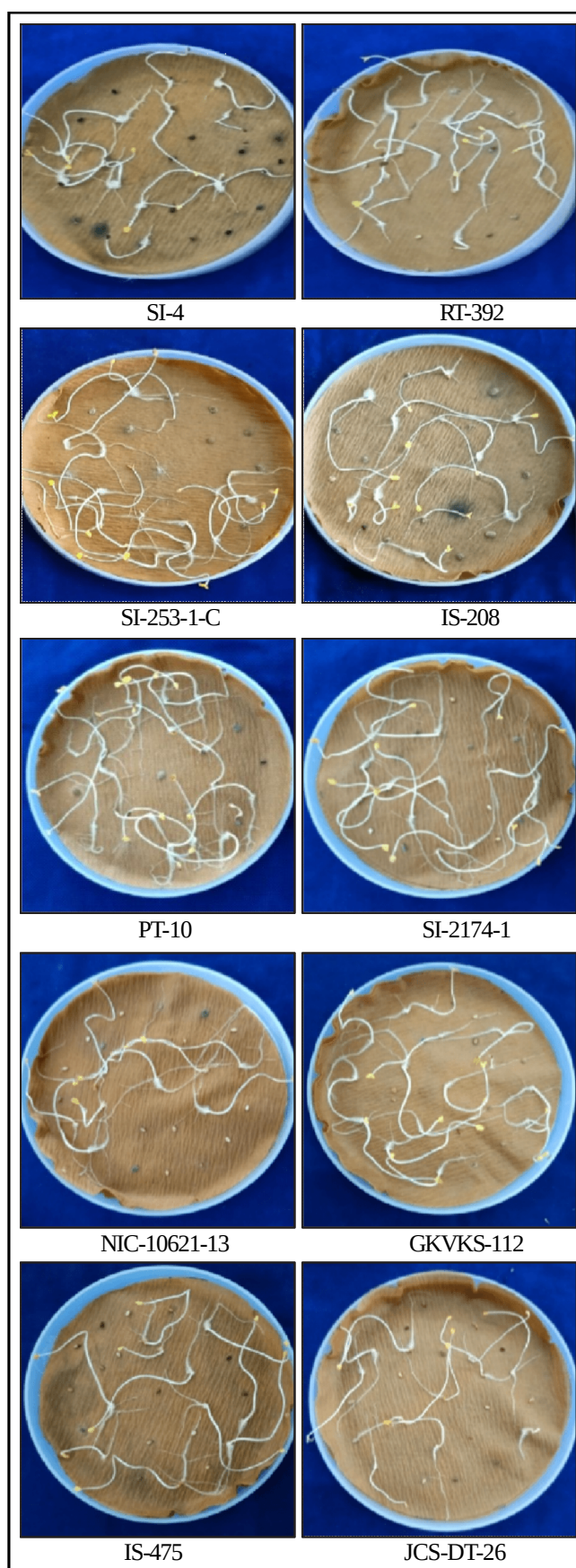


**Plate 1:** Seed health testing and seed quality parameters of susceptible genotypes using standard blotter method

**Table 1:** Seed mycoflora diversity of sesame genotypes by using standard blotter method.

| S. No.     | Sesame genotypes | <i>Alternaria</i> spp. | <i>Curvularia</i> spp. | <i>Aspergillus flavus</i> | <i>Aspergillusniger</i> | <i>Helminthosporium</i> spp. | <i>Rhizopus</i> spp. |
|------------|------------------|------------------------|------------------------|---------------------------|-------------------------|------------------------------|----------------------|
| 1          | IS-4             | 19.00                  | 0.00                   | 1.00                      | 1.80                    | 4.70                         | 0.00                 |
| 2          | RT-392           | 27.35                  | 1.00                   | 7.90                      | 0.00                    | 0.00                         | 0.00                 |
| 3          | SI-253-1-C       | 14.80                  | 0.00                   | 20.50                     | 0.00                    | 0.00                         | 0.00                 |
| 4          | IS-208           | 36.50                  | 0.00                   | 14.00                     | 0.00                    | 4.50                         | 1.50                 |
| 5          | PT-10            | 23.25                  | 0.00                   | 27.50                     | 0.00                    | 0.00                         | 0.00                 |
| 6          | SI-2174-1        | 32.12                  | 0.00                   | 15.88                     | 0.00                    | 0.00                         | 0.00                 |
| 7          | NIC-10621-13     | 24.50                  | 3.80                   | 10.20                     | 1.30                    | 2.70                         | 0.00                 |
| 8          | GKVKS-112        | 25.20                  | 0.00                   | 7.90                      | 0.00                    | 3.90                         | 0.00                 |
| 9          | IS-475           | 17.20                  | 0.00                   | 3.55                      | 0.00                    | 0.00                         | 0.00                 |
| 10         | JCS-DT-26        | 21.40                  | 0.00                   | 4.60                      | 0.00                    | 0.00                         | 0.00                 |
| Mean       |                  | 24.13                  | 0.48                   | 11.30                     | 0.31                    | 1.58                         | 0.15                 |
| Total      |                  | 241.32                 | 4.80                   | 113.03                    | 3.10                    | 15.80                        | 1.50                 |
| Percentage |                  | 63.58                  | 1.26                   | 29.78                     | 0.81                    | 4.16                         | 0.39                 |

spp., *Aspergillus niger* and *Rhizopus* spp. recorded 4.16%, 1.26%, 0.81% and 0.39% respectively (Table 1). *Alternaria* and *A. flavus* were observed in all genotypes, while *Helminthosporium* spp. appeared in four, *Curvularia* spp. and *A. niger* in two and *Rhizopus* in one genotype. *Alternaria* incidence was highest in IS-208 (36.50%) and lowest in SI-253-1-C (14.8%). *A. flavus* was more in PT-10 (27.5%) and least in IS-4 (1.0%). *Curvularia* occurred only in NIC-10621-13 (3.8%) and RT-392 (1.0%). *A. niger* was recorded in IS-4 (1.8%) and NIC-10621-13 (1.3%). *Helminthosporium* spp. incidence ranged from 4.7% (IS-4) to 2.7% (NIC-10621-13), while *Rhizopus* was confined to IS-208 (1.5%). NIC-10621-13 showed the highest fungal diversity (five fungi), followed by IS-208 and IS-4 with four fungi each.

Seed-borne fungi such as *Alternaria* spp., *Curvularia* spp., *Aspergillus flavus*, *A. niger*, *Helminthosporium* spp. and *Rhizopus* spp. are commonly

detected by the standard blotter method due to their prevalence and ability to grow on diverse substrates and are reported across crops like sunflower, maize and wheat (Addrah *et al.*, 2019; Bhattacharya and Raha, 2002; Goko *et al.*, 2021). This method supports fungal growth and sporulation, making it a simple and effective tool for detection and identification, with implications for seed quality, germination and plant health (Jeon *et al.*, 2015). Earlier studies also reported diverse fungi in sesame seeds, including *Alternaria*, *Fusarium*, *Verticillium*, *Cercospora*, *Macrophomina*, *Sclerotinia*, *Chaetomium*, *Rhizoctonia*, *Trichothecium*, *Mucor* and *Penicillium* (Nagaraj *et al.*, 2009; Lemture *et al.*, 2010; Pravallika *et al.*, 2018). In the present study, *Alternaria* spp. showed the highest incidence, reflecting field-level contamination, while *Curvularia* and *Helminthosporium* occurred as minor field fungi. *Aspergillus flavus* and *A. niger*, being storage fungi, highlight the need for better storage conditions. These findings emphasize improved crop and

**Table 2:** Identification of seed mycoflora with Agar plate method for sesame genotypes.

| S. No.     | Sesame genotypes | <i>Alternaria</i> spp. | <i>Curvularia</i> spp. | <i>Aspergillus flavus</i> | <i>Aspergillusniger</i> | <i>Helminthosporium</i> spp. | <i>Endophoma</i> spp. | <i>Rhizopus</i> spp. |
|------------|------------------|------------------------|------------------------|---------------------------|-------------------------|------------------------------|-----------------------|----------------------|
| 1          | IS-4             | 16.50                  | 0.00                   | 15.20                     | 25.24                   | 0.00                         | 0.00                  | 10.20                |
| 2          | RT-392           | 15.62                  | 0.00                   | 24.00                     | 16.20                   | 0.00                         | 3.30                  | 24.00                |
| 3          | SI-253-1-C       | 27.50                  | 0.00                   | 0.00                      | 0.00                    | 0.00                         | 0.00                  | 14.00                |
| 4          | IS-208           | 30.33                  | 0.00                   | 15.22                     | 26.50                   | 0.00                         | 0.00                  | 13.50                |
| 5          | PT-10            | 15.00                  | 4.30                   | 0.00                      | 32.00                   | 0.00                         | 0.00                  | 20.00                |
| 6          | SI-2174-1        | 28.25                  | 4.10                   | 0.00                      | 15.01                   | 4.00                         | 6.02                  | 0.00                 |
| 7          | NIC-10621-13     | 22.54                  | 0.00                   | 0.00                      | 0.00                    | 2.00                         | 0.00                  | 8.00                 |
| 8          | GKVKS-112        | 19.03                  | 6.30                   | 0.00                      | 35.00                   | 8.00                         | 2.00                  | 0.00                 |
| 9          | IS-475           | 20.70                  | 0.00                   | 14.50                     | 0.00                    | 8.00                         | 3.40                  | 23.50                |
| 10         | JCS-DT-26        | 18.13                  | 0.00                   | 0.00                      | 36.01                   | 0.00                         | 4.20                  | 12.50                |
| Mean       |                  | 21.36                  | 1.47                   | 6.89                      | 18.59                   | 2.20                         | 1.89                  | 12.57                |
| Total      |                  | 213.6                  | 14.70                  | 68.92                     | 185.96                  | 22.00                        | 18.92                 | 125.70               |
| Percentage |                  | 32.87                  | 2.26                   | 10.60                     | 28.61                   | 3.38                         | 2.91                  | 19.34                |

**Table 3:** Details of reference sequence obtained from NCBI for comparative analysis.

| S.No | Isolate                                         | Accession number | E value | (%)Similarity |
|------|-------------------------------------------------|------------------|---------|---------------|
| 1    | <i>Alternaria</i> TPT isolate                   | PX149315         | -       | -             |
| 2    | <i>Alternaria alternata</i> isolate CATAS- AA92 | ON454982.1       | 0.0     | 100           |
| 3    | <i>Alternaria alternata</i> isolate S9-2        | MW509967.1       | 0.0     | 100           |
| 4    | <i>Alternaria alternata</i> strain HDJZ-zwm- 34 | JN673372.1       | 0.0     | 100           |
| 5    | <i>Alternaria alternata</i> isolate B7-3        | MW509979.1       | 0.0     | 100           |
| 6    | <i>Alternaria alternata</i> isolate CATAS- AA91 | MZ734477.1       | 0.0     | 100           |
| 7    | <i>Alternaria alternata</i> strain ZG-4-2-4 18S | KT192411.1       | 0.0     | 100           |
| 8    | <i>Alternaria alternata</i> isolate S8-8        | MW509970.1       | 0.0     | 100           |
| 9    | <i>Alternaria alternata</i> isolate B8-1        | MW509977.1       | 0.0     | 100           |
| 10   | <i>Alternaria alternata</i> isolate M4-2        | MW509975.1       | 0.0     | 100           |
| 11   | <i>Alternaria alternata</i> isolate S8-1        | MW509972.1       | 0.0     | 100           |
| 12   | <i>Alternaria alternata</i> isolate S6-11       | MW509969.1       | 0.0     | 100           |
| 13   | <i>Alternaria alternata</i> isolate S5-5-1      | MW509966.1       | 0.0     | 100           |
| 14   | <i>Alternaria alternata</i> isolate Sample-16   | OQ421565.1       | 0.0     | 100           |
| 15   | <i>Alternaria alternata</i> isolate CATAS- AA93 | ON454983.1       | 0.0     | 100           |
| 16   | <i>Alternaria tenuissima</i> strain ZZ9-5-1     | MN907695.1       | 0.0     | 100           |
| 17   | <i>Alternaria alternata</i> isolate S7-3        | MW509971.1       | 0.0     | 100           |
| 18   | <i>Alternaria alternata</i> isolate B9-4        | MW509976.1       | 0.0     | 100           |
| 19   | <i>Alternaria alternata</i> isolate AKL Aa2     | OM976647.1       | 0.0     | 100           |
| 20   | <i>Alternaria alternata</i> isolate B1-3        | MW509978.1       | 0.0     | 100           |
| 21   | <i>Alternaria alternata</i> isolate M1-6        | MW509980.1       | 0.0     | 100           |

seed management through integrated practices such as crop rotation, certified disease-free seed, fungicide application, sanitation and storage management to reduce inoculum levels and prevent spread.

In Agar plate method on PDA revealed seven seed-borne fungi: *Alternaria* spp., *Curvularia* spp., *Aspergillus flavus*, *Aspergillus niger*, *Helminthosporium* spp., *Endophoma* spp. and *Rhizopus* spp. (Table 2). *Alternaria* spp. was detected in all genotypes with the highest incidence (32.87%), followed by *A. niger* (28.61%) and *Rhizopus* spp. (19.34%). *A. flavus* (10.60%), *Helminthosporium* (3.38%), *Endophoma* (2.91%) and *Curvularia* (2.26%) showed lower infection rates (Plate 2). Among genotypes, IS-208 showed the highest *Alternaria* incidence (30.33%), while PT-10 recorded the least (15%). *A. niger* was highest in JCS-DT-26 (36.01%) and showed no incidence in SI-253-1-C, IS-475 and NIC-10621-13. *Rhizopus* incidence was more in RT-392 (24.00%) and was absent in SI-2174-1 and GKVKS-112. *A. flavus* was more in RT-392 (24.00%), *Helminthosporium* (8%) was highest in GKVKS-112 and IS-475, *Endophoma* was highest in SI-2174-1 (6.02%) and *Curvularia* was highest in GKVKS-112 (6.30%).

In this study, *Alternaria* spp. again showed higher incidence than other fungi, similar to results from the standard blotter method. Although the agar plate method supports greater fungal growth, it carries higher

contamination risks and requires stricter sterile conditions. *Aspergillus* spp. are commonly detected in air and agricultural products, with *A. niger* (14.47%), *A. sydowii* (10.37%) and *A. flavus* (7.93%) reported as abundant airborne spores (Odeh et al., 2020). Using PDA medium, the agar plate method provides an ideal environment for fungal growth and sporulation, facilitating clear detection and identification (Addrah et al., 2019; Gonçalves et al., 2017). Similar observations on seed-borne mycoflora diversity have been reported in sesame (Nayyar et al., 2013; Bharathi et al., 2013), soybean (Rao et al., 2015; Ahmed et al., 2016), sunflower (Khalil et al., 2014; Srinivas et al., 2017; Agalave, 2018; Patil et al., 2018) and safflower (Gayathri et al., 2018).

#### Morphological identification of *Alternaria* spp.

Pure culture of *Alternaria* spp. grown on PDA media, obtained from single-spore method was used for the morphological identification (Plate 3). Colony appeared after 2-3 days and it takes 12-14 days for complete coverage of 90 mm petri plate. It produced cottony, olive-green colonies with a grayish-white center. Concentric zonation was distinct, clearly separating the central and peripheral regions. Growth was moderate and uniform across the petri plate. Colony margins were irregular and slightly lobed, resulting in a rough circular outline. Conidia appeared as dark brown coloured and conidia length ranged from 11.07- 12.55 µm and width was ranged from 4.55- 5.98 µm. Conidia showed 3-5

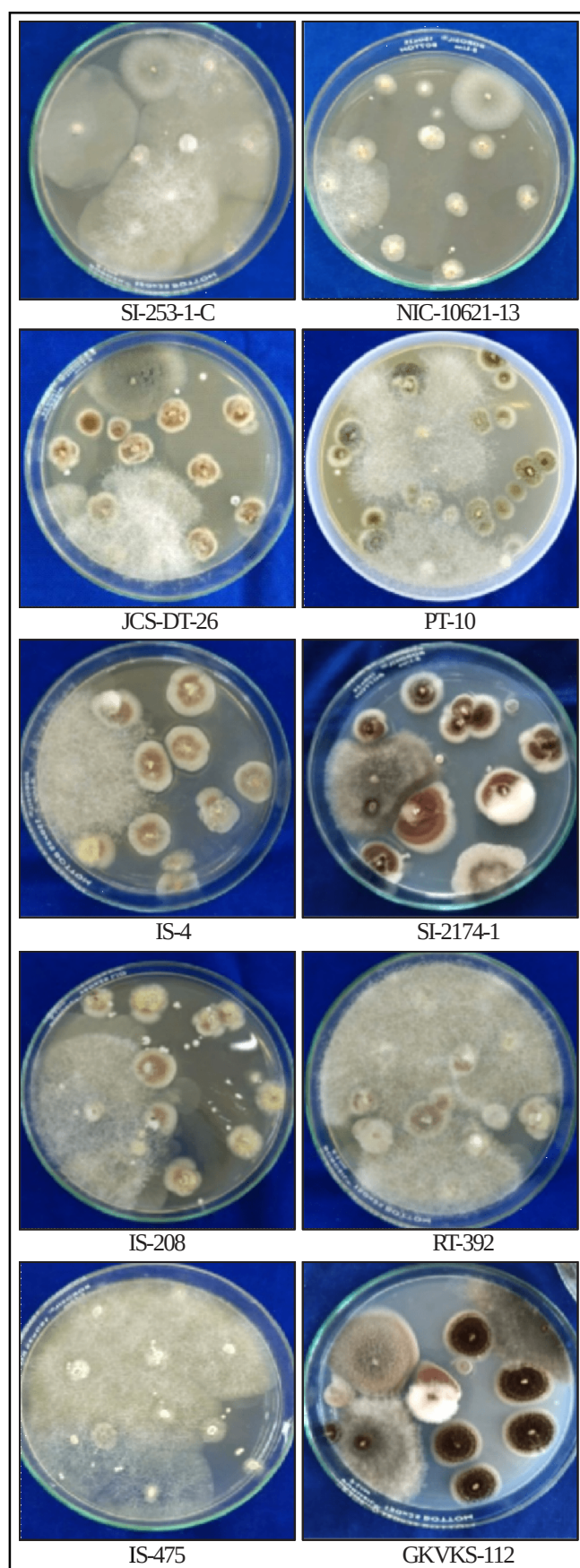


**Plate 2:** Seed borne mycoflora detected on sesame seeds using agar plate method.

longitudinal septa. Transverse septa was absent in most of conidia while in some 1-2 transverse septa was observed. Shape of the conidia was obclavate with small beak (Plate 3). The cultural and conidia conidial characteristics proved of this proved to be the pathogen as *Alternaria* genera. To confirm the species of *Alternaria* molecular characterization was carried out in the next experiment.

Morphologically, these species can be characterized based on conidial size and septation, colony color and texture, among other features. A defining trait of *Alternaria alternata* is the short or absent apical beak, which distinguishes it from large-spored species such as *A. solani*. For instance, conidial morphology is widely employed to differentiate *Alternaria solani* from *A. alternata*, despite the lack of distinct morphological characters for the small-spored species (Tymon *et al.*, 2015).

#### **Molecular characterization of *Alternaria* spp. using ITS region**

The ITS (Internal transcribed spacer) region is highly variable among different fungal species, yet conserved within species, making it an effective genetic marker for identifying and differentiating fungal species, including those within the *Alternaria* genus (Garganese *et al.*, 2018). This region provides sufficient variability to distinguish between closely related species and helps in accurately classifying them.

Total genomic DNA was extracted from the mycelial mass of pure *Alternaria* culture by using CTAB method. The extracted fungal DNA was then used as a template for polymerase chain reaction (PCR) amplification. PCR was performed using the forward primer ITS1 (52 - TCCGTAGGTGAACCTGCGG-32 ) and the reverse primer ITS4 (52 - TCCTCCGCTTATTGATATGC-32 ) for the internal transcribed spacer (ITS) region of ribosomal DNA. PCR product of approximately 550 base pairs was obtained after subjecting to agarose

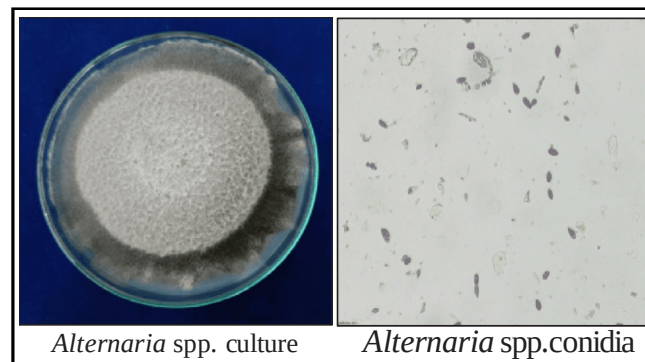


**Plate 3:** Cultural and morphological characteristics of *Alternaria* TPT isolate.

gelelectrophoresis (Plate 4). The amplified product was eluted from gel and sent for sequencing. The forward and reverse sequences trimmed with “Finish TV” software and consensus sequence was generated using clustal W option in bioedit software. Thus partial sequence of 550 bp of ITS region of *Alternaria* spp. was obtained. ITS sequences of *Alternaria* spp. isolate in this study was blasted for nucleotide similarity in NCBI database. The nucleotide similarity showed 100% sequence similarity.

Comparison of 550 base pair partial sequences of *Alternaria alternata* showed 100 % sequence similarity with *Alternaria alternata* isolate CATAS-AA92 (ON454982.1), *Alternaria alternata* isolate S9-2 (MW509967.1), *Alternaria alternata* strain HDJZ-zwm-34 (JN673372.1), *Alternaria alternata* isolate B7-3 (MW509978.1), *Alternaria alternata* isolate CATAS-

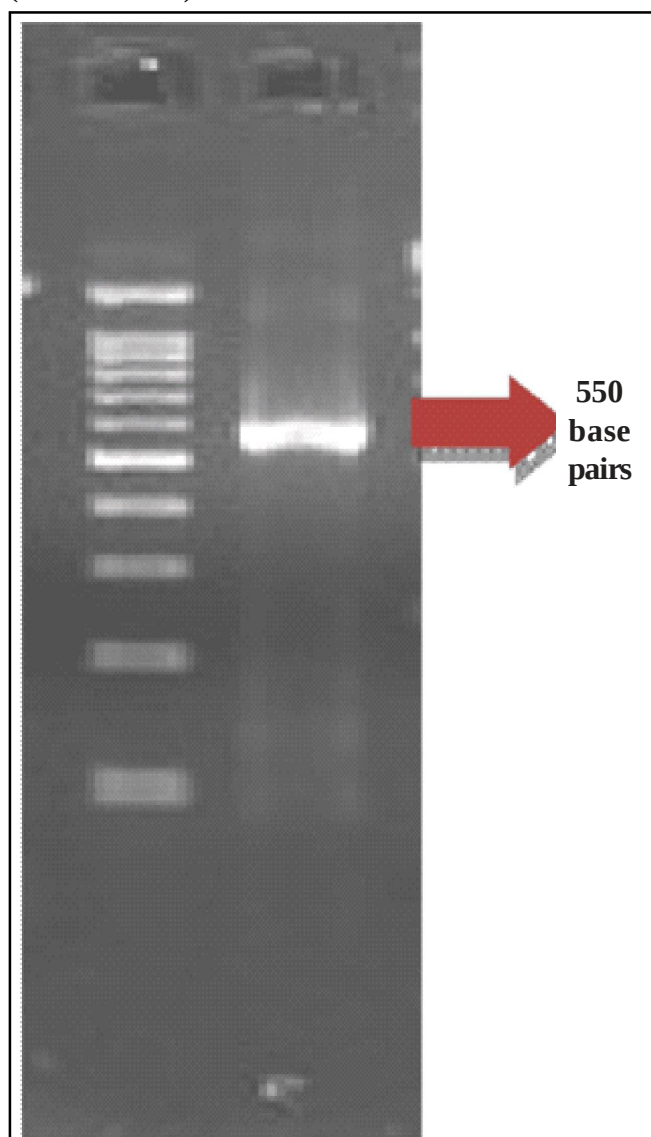


**Plate 4:** Amplified PCR product of *Alternaria* TPT isolate (ITS region) Visualized on Agarose Gel electrophoresis.

AA91 (MZ734477.1), *Alternaria alternata* strain ZG-4-2-4 18S (KT192411.1), *Alternaria alternata* isolate S8-8 (MW509970.1), *Alternaria alternata* isolate B8-1 (MW509977.1), *Alternaria alternata* isolate M4-2 (MW509975.1), *Alternaria alternata* isolate S8-1 (MW509972.1), *Alternaria alternata* isolate S6-11 (MW509969.1), *Alternaria alternata* isolate S5-5-1 (MW509966.1), *Alternaria alternata* isolate Sample-16 (OQ421565.1), *Alternaria alternata* isolate CATAS-AA93 (ON454983.1), *Alternaria tenuissima* strain ZZ9-5-1 (MN907695.1), *Alternaria alternata* isolate S7-3 (MW509971.1), *Alternaria alternata* isolate B9-4 (MW509976.1), *Alternaria alternata* isolate AKL Aa2 (OM976647.1), *Alternaria alternata* isolate B1-3 (MW509978.1), *Alternaria alternata* isolate M1-6 (MW509980.1).

Percent similarity of studied isolate *Alternaria* TPT recorded 100% match with *Alternaria alternata* respective reference strains (Table 3). Thus this study confirms that the pathogen associated with leaf blight symptoms and seed infestation is *Alternaria alternata*.

#### Partial sequence of *Alternaria alternata* isolate submitted to gene bank

CCGTAGGGTGAACCTGCGGAGGGATCATTACA

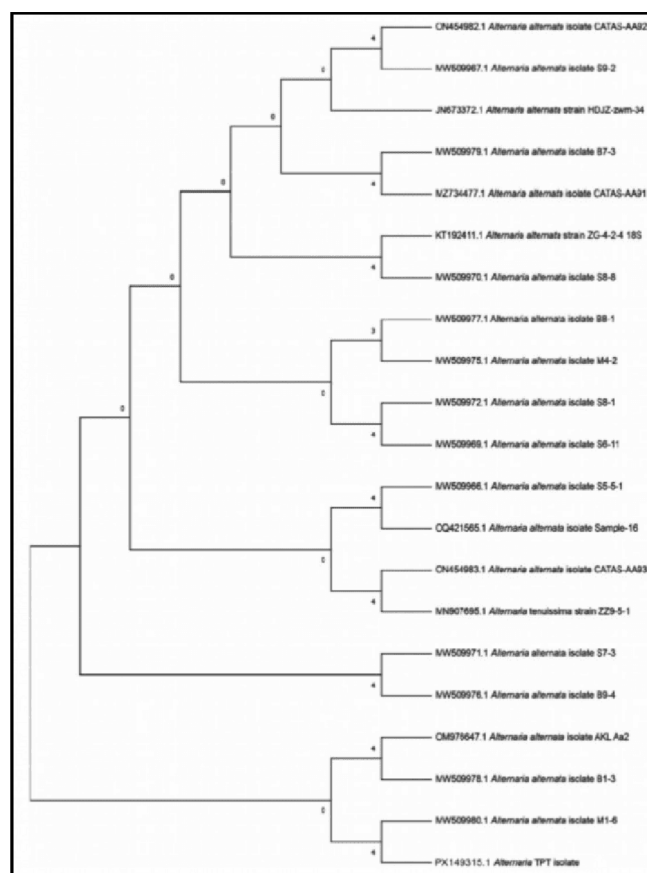


**Fig. 1:** Phylogenetic tree was constructed using Mega 11 software with 1000 bootstrap replications.

CAAATATGAAGGCGGGCTGGAACCTCTCGGGG  
 TTACAGCCTTGCTGAATTATTCACCCTTGCTCT  
 TTTGCGTACTTCTTGTTCCTTGGTGGGTTCGCC  
 CACCCTAGGACAAACATAAACCTTTTGTAA  
 TTGCAATCAGCGTCAGTAACAAATTAATAATTA  
 CAACCTTTCAACAACGGATCTCTTGGTTCTGGC  
 ATCGATGAAGAACGCAGCGAAATGCGATAAG  
 TAGTGTGAATTGCAGAAATTCAGTGAATC  
 ATCGAATCTTTGAACGCACATTGCGCCCTTTGGT  
 ATTCCAAAGGGCATGCCTGTTTCGAGCGTCATTGTAC  
 CCTCAAGCTTTGCTTGGTGTGGGCGTCTTGTC  
 TCTAGCTTTGCTGGAGACTCGCCTTAAAGTAA  
 TTGGCAGCCGGCCTACTGGTTTCGGAGCGC  
 AGCACAAGTCGCACTCTCTATCAGCAAAGG  
 TCTAGCATCCATTAAGCCTTTTTTCAACTTTTG  
 ACCTCGGATCAGGTAGGGATACCCGCTGAAC  
 TTAAGCATATCAATAAGCCGGAGG.

### Phylogenetic tree analysis

A total of 20 reference sequences retrieved from GenBank, were subjected to molecular phylogenetic analysis using MEGA11 software with bootstrap values 1000 using maximum likelihood method. The phylogenetic tree was constructed based on nucleotide sequences to evaluate the evolutionary relationships among the isolates (Fig. 1).

The test sample grouped closely with other *Alternaria alternata* strains, particularly those with GenBank accession numbers MW509980.1 (isolate M1-6), MW509978.1 (isolate B1-3) and MW509976.1 (isolate B9-4), indicating high sequence similarity and suggesting a common evolutionary lineage. These groupings were supported by high bootstrap values, confirming the reliability of the clustering.

Isolates such as ON454982.1 (CATAS-AA92), MW509967.1 (S9-2) and JN673372.1 (HDJZ-zwm-34) formed a separate clade, indicating slight genetic divergence from the main cluster. Meanwhile, *Alternaria tenuissima* (MN907695.1) distinctly branched away from all *A. alternata* isolates, serving as an outgroup in the tree.

The constructed phylogenetic tree effectively demonstrated the genetic closeness and diversity among the *A. alternata* isolates analyzed. The clustering pattern also supports the identification of the test sample as *Alternaria alternata* and helps infer its phylogenetic relationship with previously reported isolates.

Nayyar *et al.*, (2017) isolated 428 *Alternaria* strains from 105 sesame seed samples, grouping them into 36 taxonomic types, with isolates A13, A47 and A215 identified as *A. alternata* via ITS rDNA sequencing and

confirmed pathogenic on sesame. Within *Alternaria* sect. *Alternaria*, species show high genetic similarity, with whole-genome identities of 96.7–98.2%, making it difficult to separate *A. alternata* from related species like *A. tenuissima* and *A. arborescens* (Woudenberg *et al.*, 2015; Wang *et al.*, 2021).

Due to limited phylogenetic variation, some researchers suggest collapsing small-spored taxa into *A. alternata*. Wang *et al.*, (2019) distinguished *A. tenuissima* (83.5%) and *A. alternata* (16.5%) in sunflower using ITS and histone 3 gene sequences, with histone 3 proving more reliable. Prasad *et al.* (2020) grouped *A. helianthi* and *A. alternata* into eleven clades based on ITS, while Haque and Parvin (2021) identified *A. alternata* isolate ColPat-420 using ITS and RPB2 sequencing.

### Conclusion

The study confirmed *Alternaria alternata* as the predominant seed-borne fungus in sesame, detected at higher incidence than other fungi by both blotter and agar plate methods. Morphological traits and ITS1/ITS4 primer-based sequencing showed 100% similarity with reference isolates, validating its identity. These findings highlight the seed-borne nature of *A. alternata* and stress the need for integrated seed health management to reduce inoculum levels and ensure healthy sesame cultivation.

**Conflict of interest:** No conflict of interest

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