**Seed health techniques for detection and management of *Alternaria* spp. in sesame**

**Abstract**

Sesame (*Sesamum indicum* L.) is a vital oilseed crop, renowned for its high protein content, superior oil quality, and antioxidant properties, and is extensively cultivated across India and Asia. However, the productivity and quality of sesame are severely affected by Alternaria leaf spot, primarily caused by *Alternaria sesami* and *Alternaria alternata*. The disease leads to significant yield losses, as seed infection correlates directly with reduced germination, seedling stand, and crop yield, with losses ranging from 4% to 25% depending on the severity. Different stages of plant growth exhibit varying susceptibility, with the greatest vulnerability occurring in plants inoculated at 8 to 12 weeks. Furthermore, *Alternaria* species, including *A. sesami* and *A. sesamicola*, are major seed-borne pathogens, with infection levels as low as 2% potentially causing substantial losses. ; To manage these risks, a variety of seed treatment methods, including fungicides, biocontrol agents, and plant extracts, have been explored, demonstrating promising results in controlling disease incidence and improving seed quality (Bhattiprolu *et al.*, 2023. Several seed health testing methods, including the standard blotter method, deep freezing blotter method, and PCR-based molecular techniques, offer effective means for detecting seed-borne *Alternaria* species. Molecular methods, particularly PCR and LAMP assays, provide rapid, sensitive, and specific detection of *Alternaria* species, enabling early diagnosis and better disease management. Additionally, serological methods like ELISA have shown potential in pathogen detection, although molecular techniques are preferred for their higher sensitivity and specificity. This review underscores the importance of effective monitoring and management strategies to mitigate the impact of *Alternaria* diseases on sesame production, highlighting the relevance of diverse diagnostic approaches in managing this crop pathogen.

**Keywords:** Alternaria, *Sesamum indicum* L.,seed health testing methods**,** productivity

**Introduction**

Sesame (*Sesamum indicum* L.) is an ancient and important oilseed crop, valued for its rich protein content, high-quality seed oil, and antioxidant properties (Mahalakshmi, 2020; Kumar et al. 2024). It is extensively cultivated in India and other parts of Asia, making it a significant agricultural commodity (Maiti *et al.*, 1985; Nayyar *et al.*, 2017). Alternaria leaf spot is a significant cause of yield losses in sesamum (*Sesamum indicum* L.). This fungal disease, primarily caused by *Alternaria sesami* and *Alternaria alternata*, can have severe impacts on crop productivity and quality. The disease severity of Alternaria leaf spot in sesamum is directly correlated with yield losses. Studies have shown that disease severity increases with higher levels of seed infection, leading to reduced seed yield, lower 1000-seed weight, and fewer seeds per capsule (Ojiambo *et al.*, 2000). The susceptibility of sesamum plants to Alternaria leaf spot varies with plant age. Plants inoculated at 8 and 12 weeks of age were found to be most susceptible to the disease, while those inoculated at 4 weeks exhibited the least susceptibility (Ojiambo *et al.*, 1999; Issahaku et al. 2023). In field experiments, yield losses due to seed infection ranged from 4% to 25%, with yields decreasing from 312.5 kg ha−1 in control plots to as low as 234.9 kg ha−1 in heavily infected plots (Ojiambo *et al.*, 2000). Interestingly, the tolerance level of *Alternaria sesami* in sesame seed was determined to be less than 2% (Ojiambo *et al.*, 2000). This low tolerance threshold emphasizes the importance of managing seed infection to prevent significant yield losses. Additionally, the disease can spread rapidly in the field, with infection rates and areas under disease progress curves (AUDPC) varying among different seed infection levels (Ojiambo *et al.*, 2000).

*Alternaria* belongs to the family *Dematiaceae* of the order *Moniliales*. The genus *Alternaria* has conidia that are septate transversely and somewhat longitudinally. They can be found individually or in chains on short or long, stiff or weak, branching or unbranched conidiophores. *Alternaria* species, particularly *A. sesami* and *A. sesamicola*, are significant seed-borne pathogens of sesame (*Sesamum indicum* L.). Studies have shown that these fungi can infect sesame seeds at varying levels, with *A. sesamicola* being the predominant species in some Korean samples, infecting up to 68% of seeds (Seung-Hun *et al.*, 1982). In eastern Kenya, seed infection levels of *A. sesami* ranged from 0 to 8%, with higher infection levels leading to increased disease severity in the field (Ojiambo *et al.*, 2000). Interestingly, *A. longissima*, previously considered a saprophyte, was found to be pathogenic on sesame, causing zonate leaf spot, foliage blight, stem necrosis, and capsule spots (Seung-Hun *et al.*, 1982). This highlights the potential for new or overlooked *Alternaria* species to emerge as seed-borne pathogens in sesame. In western Kenya, seed infection levels of *A. sesami* varied from 9% to 24% across different districts (Ojiambo *et al.*, 2000). The seed-borne nature of *Alternaria* in sesame significantly impacts crop health and yield. Seed infection levels as low as 2% can lead to reduced germination, seedling stand, and yield losses ranging from 4% to 25% (Ojiambo *et al.*, 2000; Pravallika *et al.*, 2021). To manage this issue, various control measures have been studied, including seed treatments with fungicides, biocontrol agents, and plant extracts (Bhattiprolu *et al.*, 2023; Lubaina & Murugan, 2013; Mahalakshmi, 2020). These treatments have shown promising results in reducing disease incidence and improving seed quality, highlighting the importance of addressing the seed-borne nature of *Alternaria* in sesame production.

**Seed health testing methods**

**Standard blotter method**

Blotter method is employed for the detection and identification of mycroflora associated with sesame seeds (ISTA 1976). The blotter method was developed by Doyer in 1938 which was later included in the International seed testing association (ISTA) rules 1966**.** Four hundred seeds of sesame are placed at the rate of 20 seeds per Petri plate on moistened blotters as described under standard blotter method. Such seeds were examined under stereoscopic-binocular microscope for the infection of *A. sesami*.Bretag and Mebalds (1987) isolated surface-borne saprophytes viz., *Alternaria alternata*, from chickpea seed by employing standard blotter method. Similarly, research in Sialkot, Pakistan employed the blotter paper method alongside agar plate and deep freezing techniques to isolate 36 fungal species from sesame seeds, with *Alternaria* being one of the predominant genera (Nayyar, 2013).

**2, 4-D Method**

The method has been recommended by ISTA (1966) as a standard test for a number of seed borne pathogens. The use of 2, 4-D in the blotter test was first introduced by Neergaard (1973) while testing cabbage seeds for *Phoma lingam.* The 2,4-D blotter method is mentioned as one of the techniques used for detecting seed-borne fungi in cowpea seeds (Zanjare *et al.*, 2020). This method was found to be less effective compared to the standard blotter paper method and agar plate method for detecting seed-borne fungi, including *Alternaria* *alternata.*

**Deep freezing blotter method**

The deep-freezing blotter method has proven to be an important technique for detecting *Alternaria* species in various seed samples. This method is particularly effective for identifying seed-borne fungi, including *Alternaria* alternata*, A. radicina*, and *A. dauci*, which are significant pathogens in carrot seeds (Konstantinova *et al.*, 2002). Deep freeze blotter method was advocated for evaluation and identification of fungus as an alternative to 2, 4-D blotter method, since this is known to suppress germination (Limonard, 1968). Compared to other detection methods, the deep-freeze blotter test has shown superior performance in several studies. For instance, in caraway seed health testing, it yielded the greatest seed infestation with fungi, especially *Alternaria* spp., followed by the blotter test with mannitol (Tylkowska *et al.*, 2015). Similarly, for milk thistle seeds, the deep-freeze blotter test was recommended along with other blotter tests for detecting important fungi, including *Alternaria* *alternata* (Rosińska *et al.*, 2013). However, it's worth noting that the effectiveness of the deep-freezing blotter method may vary depending on the seed type and target fungi. In some cases, other methods have shown comparable or better results. For example, in cowpea seed testing, the standard blotter paper method was found to be more effective than the deep freeze blotter paper method (Zanjare *et al.*, 2020).

**Agar plate method**

In Northern Ireland, Muskett and Malone (1941) first time used this method for seed health testing of flax seeds. Agar plate method effectively detected *Alternaria sesami* infection in sesame seeds collected from farmers in western Kenya, with infection levels ranging from 9% to 24% (Ojiambo *et al.*, 2000). The identification of *Alternaria sesami* was based on characteristic growth colonies produced on oat meat agar method. The per cent seed infection by *A. sesami* in harvested seed was assessed using the oat meal agar method (Neergard, 1979).Jeffrey *et al.* (1985) suggested the modified agar plate method for the detection of *Alternaria helianthi* (Hansf.) Tubaki and Nishin, in sunflower and the whole mount method was advocated by Krishnappa and Shetty (1990) for detection of the pathogen. This method allowed researchers to quantify infection levels and study their impact on disease severity in field conditions. Similarly, the agar plate method was used to detect various seed mycoflora, including *Alternaria alternata*, in Indian bean cultivars (Prajapati & Patel, 2020). In a comparative study of detection methods for sesame seed mycoflora in Pakistan, the agar plate method yielded 22 fungal species, including *Alternaria*, demonstrating its effectiveness in identifying a wide range of fungi (Nayyar, 2013). However, it's worth noting that in some studies, the blotter method outperformed the agar plate method in detecting seed mycoflora. For instance, in safflower seeds, the blotter method detected a higher number of fungi compared to the agar plate method (Gayathri & Rao, 2018). In conclusion, the agar plate method is a valuable tool for detecting *Alternaria* in sesame seeds, allowing researchers to quantify infection levels and study their impact on crop health and yield. While it may not always be the most sensitive method, it remains an important technique in seed health testing and mycoflora detection.

**Paper towel method**

Paper towel method is one of several techniques used to detect *Alternaria* in sesame seeds. According to Gupta *et al.* (2023), the paper towel method, along with standard blotter method and standard agar plate method, was employed to detect seed mycoflora in 165 genotypes of sesame. *Alternaria* was among the six distinct fungi identified using these methods (Gupta *et al.*, 2023). Interestingly, while the paper towel method was effective in detecting *Alternaria*, the standard blotter method identified the maximum number of seeds infected by fungi compared to the paper towel and standard agar plate methods (Gupta *et al.*, 2023). This suggests that different detection methods may have varying sensitivities for identifying seed-borne pathogens in sesame. In conclusion, the paper towel method is a viable technique for detecting *Alternaria* in sesame seeds, as demonstrated in Gupta *et al.* (2023). However, it's important to note that other methods, such as the standard blotter method, may be more effective in identifying a broader range of fungal infections. The choice of detection method may depend on the specific research object.**Serological methods**

Serological methods such as various type of ELISA have shown to be increased sensitivity and accuracy for detecting *Alternaria* species compared to traditional isolation techniques (Nagrale *et al.*, 2016). These immunological assays provide a more rapid and precise approach to identifying pathogenic *Alternaria* in crops like sesame. Enzyme-linked immunosorbent assay (ELISA) has proven to be a highly sensitive and effective serological method for detecting various fungal pathogens in crops, including *Alternaria* species. While the provided context does not specifically mention the detection of *Alternaria* in sesamum crops, it offers valuable insights into the application of ELISA for fungal detection in various plant systems (Barker & Pitt, 1988; Ivanova *et al.*, 2020; Musgrave, 1984). Notably, Ivanova *et al.* (2020) specifically mentions the effectiveness of ELISA methods for detecting *Alternaria alternata*, among other soil microfungi. The competitive ELISA method was found to be more efficient than the two-site sandwich ELISA for this purpose. Interestingly, the sensitivity of ELISA can vary depending on the specific application and target organism. For instance, in the detection of Colletotrichum sp. in anemone tissue, the lower detection limit was less than 100 ng/ml of dry weight pure mycelium. Interestingly, while serological methods like ELISA and immunofluorescence tests are commonly used for detecting other pathogens like Chlamydia and Trichinella (Bruschi *et al.*, 2019; Russell, 1999), their application for *Alternaria* detection is not extensively discussed in the provided context. This suggests that molecular techniques may be preferred for *Alternaria* identification due to their higher sensitivity and specificity. While serological methods have been used for *Alternaria* toxin detection, PCR-based techniques appear to be more sensitive and widely adopted for *Alternaria* species identification in plants and food samples.

**Molecular methods**

Molecular methods, particularly PCR-based techniques, have emerged as powerful tools for the detection of *Alternaria* species in various crops, including sesamum. These methods offer rapid, sensitive, and specific detection compared to traditional culture-based approaches. For sesame specifically, a study conducted in Pakistan utilized ITS sequencing and the *Alternaria* major allergen gene (Alt a 1) to identify *Alternaria alternata* as a major cause of leaf blight disease in sesame crops (Nayyar *et al.*, 2017). The Alt a one sequences showed >99% homology with *A. alternata*, providing a reliable molecular marker for detection. A study reported the development of a PCR assay using primers designed to target the noxB gene, which successfully detected *Alternaria* species in nine different crop plants (Chakdar *et al.*, 2019). In another study, a PCR method targeting the ITS regions of the rRNA gene was developed for rapid identification of *Alternaria* spp (Pavón et al. 2012a). Another research utilized primers targeting the internal transcribed spacer (ITS) regions of the 5.8S rRNA gene for *Alternaria* detection in tomato products (Zur *et al.*, 1999). While these studies did not specifically mention sesame, the principles could potentially be applied to detect *Alternaria* in sesamum crops as well. Interestingly, some studies have explored more advanced molecular techniques for *Alternaria* detection. For example, a loop-mediated isothermal amplification (LAMP) assay targeting the cytochrome b gene was developed for rapid and sensitive detection of *Alternaria* species (Yang *et al.*, 2019). Real-time PCR methods have also been established, offering quantitative estimation of seed infection (Guillemette *et al.*, 2004 Interestingly, while molecular methods offer advantages in speed and sensitivity, some studies have explored alternative approaches. For instance, research on Mikania scandens plant extract as a potential biocontrol agent against *Alternaria* leaf spot in sesame demonstrated that biochemical changes in treated plants could be used as indicators of pathogen presence and plant defense response (Lubaina & Murugan, 2013). Molecular methods, particularly PCR-based assays, offer rapid detection of plant samples. The development of rapid, sensitive, and specific molecular diagnostic tools has largely superseded serological methods for *Alternaria* detection in most applications (Pavón et al. 2012b, Chakdar *et al.*, 2019; Leiminger *et al.*, 2014; Nagrale *et al.*, 2016; Özer & Bayraktar, 2019; Pavón *et al.*, 2011; Zur *et al.*, 2002).

Are serological and molecular techniques better than physical and culture methods? It would be better to mention their merits and demerits?

**Conclusion**

In conclusion, seed health testing plays a crucial role in identifying and managing seed-borne pathogens, such as *Alternaria* species, which are significant for crops like sesame. A variety of methods are available, each with its strengths and limitations. The **standard blotter method** remains one of the most widely used and effective techniques for detecting fungal infections, including *Alternaria*. However, alternative approaches like the **2, 4-D method**, **deep-freezing blotter method**, and **agar plate method** offer useful variations for specific fungi and seed types, with some methods providing superior sensitivity under certain conditions. The **paper towel method** shows potential as a simple and effective detection technique, but it may not always outperform the standard blotter method. **Serological methods**, such as ELISA, have also been employed to detect fungal pathogens, but they are generally less sensitive than molecular methods. **Molecular methods**, particularly PCR-based techniques, have revolutionized seed health testing by providing rapid, highly sensitive, and specific detection of pathogens like *Alternaria*. These molecular assays are increasingly preferred due to their speed and accuracy, although they often require specialized equipment. Despite the advancements in molecular and serological techniques, a **polyphasic approach** combining traditional, molecular, and biochemical methods is often recommended for comprehensive and reliable pathogen detection. Overall the choice of seed health testing method depends on factors like the target pathogen, seed type, available resources, and research objectives. Advances in molecular diagnostics, however, are shaping the future of seed health testing, offering more efficient and precise tools for managing plant diseases. Research gap??????????????

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