Journal of Agricultural Faculty of Gaziosmanpasa University
Gaziosmanpaşa Üniversitesi Ziraat Fakültesi Dergisi
https://dergipark.org.tr/tr/pub/gopzfd

Research Article

JAFAG (2025) 42(2), 89-94
ISSN: 1300-2910
E-ISSN: 2147-8848
DOI: 10.55507/gopzfd.1648622

Detection and molecular characterization of *Macrophomina phaseolina* isolates in sesame using species-specific markers*

Amjad ALI[1]*, Muhammed TATAR[2], Fatih ÖLMEZ[1], Muhammad Azhar NADEEM[3], Faheem Shahzad BALOCH[3,4]

[1]Sivas University of Science and Technology, Faculty of Agricultural Sciences and Technologies, Department of Plant Protection, Sivas 58140, Türkiye
[2]Sivas University of Science and Technology, Faculty of Agricultural Sciences and Technologies, Department of Field Crops, Sivas 58140, Türkiye
[3]Mersin University, Faculty of Science, Department of Biotechnology, Yenişehir, Mersin 33343, Türkiye
[4]Jeju National University, Departmen of Plant Resources and Environment, 63243, Republic of Korea

***Corresponding author e-mail:** amjadbzu11@gmail.com

*This study is part of the Ph.D. thesis of Amjad Ali, the first author, accepted by the Graduate School of Sivas Science and Technology University.

Received: 28.02.2025 Accepted: 14.04.2025

Abstract: Sesame (*Sesamum indicum* L.) is a vital oilseed crop cultivated globally, yet its production is significantly threatened by charcoal rot disease caused by *Macrophomina phaseolina*. This soilborne pathogen leads to severe yield losses, affecting sesame-growing regions globally. In this study, we conducted a molecular characterization of *M. phaseolina* isolates collected from infected sesame plants in Diyarbakır and Şanlıurfa, Türkiye. During the field survey, the characteristic symptoms such as wilting, stunted growth, and blackened stems were observed and samples were collected to isolate the targeted pathogen. Seven isolates were successfully isolated and analyzed for their morphological and microscopic features, confirming the presence of *M. phaseolina*. The DNA extraction and PCR amplification using species-specific primers (MpKRI/MpKFI) successfully detected the pathogen in all isolates, producing a distinct 350 bp amplicon. The results validate the reliability of molecular markers in identifying *M. phaseolina* with high specificity. This study highlights the importance of molecular tools for precise pathogen detection, aiding in disease management strategies. The findings contribute to a better understanding of *M. phaseolina*'s genetic diversity and offer valuable insights for breeding programs aimed at developing resistant sesame cultivars. Future research should focus on integrating molecular diagnostics with breeding efforts to enhance sesame's resilience against charcoal rot disease.

Keywords: Fungal pathogens, *Macrophomina phaseolina*, molecular characterization, sesame breeding, *Sesamum indicum*.

Türlere özgü markörler kullanılarak susamda *Macrophomina phaseolina* izolatlarının tespiti ve moleküler karakterizasyonu

Öz: Susam (*Sesamum indicum* L.), dünya genelinde yetiştirilen önemli bir yağ bitkisi olup, üretimi Macrophomina *phaseolina*'nın neden olduğu kömür çürüklüğü hastalığı tarafından ciddi şekilde tehdit edilmektedir. Toprak kökenli bu patojen, susam yetiştirilen bölgelerde önemli verim kayıplarına yol açmaktadır. Bu çalışmada, Diyarbakır ve Şanlıurfa, Türkiye susam üretim alanlarındaki enfekte susam bitkilerinden izole edilen *M. phaseolina* izolatlarının moleküler karakterizasyonu gerçekleştirilmiştir. Arazi çalışmaları sırasında solgunluk, bodur büyüme ve siyahlaşmış gövdeler gibi karakteristik semptomlar tespit edilmiştir. Yedi izolat başarıyla elde edilerek morfolojik ve mikroskobik özellikleri incelenmiş ve *M. phaseolina*'nın varlığı doğrulanmıştır. Türlere özgü primerler (MpKRI/MpKFI) kullanılarak gerçekleştirilen DNA ekstraksiyonu ve PCR amplifikasyonu, tüm izolatlarda patojeni başarıyla tespit etmiş ve 350 bp'lik özgün bir amplikon ürünü üretmiştir. Sonuçlar, moleküler markörlerin *M. phaseolina*'nın yüksek özgüllükle tanımlanmasındaki güvenilirliğini doğrulamaktadır. Bu çalışma, hastalık yönetim stratejilerine katkı sağlamak için moleküler araçların hassas patojen tespitindeki önemini vurgulamaktadır. Bulgular, *M. phaseolina*'nın genetik çeşitliliğinin daha iyi anlaşılmasına katkıda bulunarak, kömür

To cite this article: Ali, A., Tatar, M., Ölmez, F., Nadeem, M.A., & Baloch, F.S. (2025). Detection and molecular characterization of *Macrophomina phaseolina* isolates in sesame using species-specific markers. *Journal of Agricultural Faculty of Gaziosmanpasa University*, 42(2), 89-94. https://doi.org/10.55507/gopzfd.1648622

çürüklüğüne dayanıklı susam çeşitlerinin geliştirilmesine yönelik ıslah programları için değerli bilgiler sunmaktadır. Gelecek araştırmalar, susamın kömür çürüklüğüne karşı direncini artırmak amacıyla moleküler tanı yöntemleri ile ıslah çalışmalarının entegrasyonuna odaklanmalıdır.

Anahtar kelimeler: Fungal patojenler, *Macrophomina phaseolina*, moleküler karakterizasyon, susam ıslahı *Sesamum indicum*.

1. Introduction

Sesame (*Sesamum indicum* L.), a member of the Pedaliaceae family, is considered one of the oldest cultivated oilseed crops, with its origins traced to Africa (Ram et al., 1990). This crop is cultivated mainly for its seeds and the oil they contain. Sesame seeds are composed of 60% oil, 25% protein, and methionine (Nayar & Mehra, 1970). Sesame is extensively cultivated in warm and temperate regions of 69 countries, as it requires 110 to 150 frost-free days for optimal growth. The leading producers include Sudan, India, Tanzania, Myanmar, and Nigeria, with production volumes of 1,119,026, 817,000, 700,000, 641,729, and 440,000 tonnes, respectively. Türkiye ranks 34th globally in sesame production, with a total production of 17,657 tonnes (FAO, 2023). Sesame breeding efforts have been undertaken in Türkiye (Yılmaz, 2022), yet production is significantly hindered by several reasons. Furthermore, it is recognized for its resilience to drought and ability to thrive in arid and semi-arid regions. Its deep root system and efficient water use make it a suitable crop for regions with limited rainfall.

Despite its inherent tolerance, climate change poses significant challenges to sesame production. Rising temperatures, erratic rainfall patterns, and prolonged droughts can affect seed germination, flowering, and overall yield. High temperatures during the reproductive stage may lead to floral abortion, reduced seed filling, and lower oil content. Additionally, changes in humidity and precipitation patterns can create favorable conditions for various pathogens, including *Macrophomina phaseolina*, the causal agent of charcoal rot disease, which significantly impacts sesame yield. As climate variability continues to intensify, understanding the interaction between environmental stressors and disease pressure is essential for developing climate-resilient sesame varieties and improving management strategies (Mahdy et al., 2023; Mahmoud et al., 2024).

M. phaseolina is a widely distributed fungus affecting >500 plant species (Sarkar et al., 2014), with sesame being one of its economically important hosts. Disease incidence in sesame fields can reach up to 39% (Balabaskar et al., 2015), causing substantial yield losses ranging between 30% and 75%. This soilborne fungus overwinters in the soil or plant debris and infects plants through roots and crown. The infection results in the formation of black microsclerotia within the vascular bundles and along the stem, enabling the pathogen to persist in the soil for over a decade, making its management particularly challenging (Gupta et al., 2020). Several strategies have been explored for managing *M. phaseolina* in sesame cultivation, including seed treatments, fungicides, soluble silicon application, plant extracts, biofumigation, intercropping, biological control, and the development of resistant host varieties. Despite these efforts, limited information is available on the disease epidemiology in Türkiye. Therefore, characterizing *M. phaseolina* isolates could provide valuable insights into disease spread and effective management strategies for charcoal rot in sesame. Molecular characterization of *M. phaseolina* pathogen open a new avanu to control the spread of virulence strains of this pathogen in different regions of the globe. Utilizing the fungus-specifc primers ensured accurate identification under laboratory conditions. During this study, we optimized species-specific primers for *M. phaseolina* in our laboratory to facilitate its molecular detection.

2. Materials and Methods

2.1. Collection of sesame diseased samples

A survey was conducted in the sesame-growing regions of Diyarbakır and Şanlıurfa in August 2024 to collect the infected root and shoot samples exhibiting characteristic symptoms of *M. phaseolina* infection. The samples were carefully collected, placed in polyethylene bags, and transported to the Plant Protection Laboratory at Sivas Science and Technology University for further processing.

2.2. PDA preparation and incubation of the diseased samples

Potato dextrose agar (PDA) was prepared by adding 39 g of commercially available medium powder which

contained 4 g of potato starch, 15 g of agar and 20 g of dextrose. This medium powder was added in one liter of distilled water and shaken thoroughly to dissolve it. After that, the pH was adjusted to 5.6, and the mixture was stirred thoroughly to ensure complete dissolution. The prepared medium was then autoclaved at 121°C for 15 minutes. Diseased samples were washed with 0.3% sodium hypochlorite, rinsed three times with distilled water, and carefully placed onto the poured PDA plates. The plates were incubated at 30°C for five days. A single-spore colony was isolated on water agar and subsequently transferred to fresh PDA plates for further characterization and DNA isolation.

2.3. Pathogenicity test

The pure culture of *M. phaseolina* was used to confirm the pathogenicity test. The suspension was prepared by adding 5 mL of autoclaved distilled water to a 14-day-old pure culture of *M. phaseolina*. This suspension was then added to a 5 kg autoclaved wheat bottle to promote the growth of *M. phaseolina*. After successful growth on the wheat seeds, the infected seeds were mixed with autoclaved peat-moss soil for 10 days to facilitate the transfer and establishment of the pathogen in the soil. After 10 days, the wheat plants were removed, and sesame seeds were sown in the filled tubes under controlled conditions at an adjusted temperature of 30°C. The infection in the treated plants was observed and compared to the control plants

2.4. DNA isolation of single spore colony growth of *M. phaseolina*

The targeted fungal growth was observed on the 10th day of incubation. Spores were scraped from the media and transferred into sterile 2 mL Eppendorf tubes. To each tube, 4–5 sterile stone beads were added to facilitate sample homogenization. DNA extraction was carried out using the CTAB protocol recommended by Doyle and Doyle (1990). A fresh buffer was prepared by mixing extraction buffer, lysis buffer, sarcosyl, PVP-40, and sodium disulfate. The buffer was preheated at 65°C for 30 minutes, after which 1 mL was added to each sample tube, followed by a second round of homogenization using a tissue homogenizer. The subsequent recommended steps were followed for DNA isolation. At the final stage, 70% ethanol was used to wash the pellet, which was then left overnight to dry. The next day, 100 µL of DNA/RNA-free molecular-grade distilled water was added to dissolve the DNA carefully. The concentration of the extracted DNA was then quantified using a NanoDrop spectrophotometer.

2.5. PCR amplification and thermocycle conditions

The PCR amplification of extracted fungal DNA was carried out using fungal-specific primers. The reaction was set up in a total volume of 10 µL, consisting of 5 µL of Vazemy enzyme P-525, 0.5 µL of each primers MpKRI/MpKFI (CGTCCGAAGCGAGGTGTATT / CCGCCA GAGGACTATCAAAC) and, 3 µL of distilled water, and 1 µL of stock DNA. The PCR thermocycling conditions were optimized based on the enzyme used, consisting of an initial denaturation at 95°C for 3 minutes, followed by 40 cycles of denaturation at 95°C for 15 seconds, annealing at 58°C for 20 seconds, and extension at 72°C for 45 seconds, with a final extension at 72°C for 6 minutes. The PCR products were electrophoresed on a 2% agarose gel at 100 V for 95 minutes.

3. Results

3.1. Observed symptoms in field

In the year 2024, a field survey was conducted in two sesame cultivation areas located in the Diyarbakır and Şanlıurfa district to assess the occurrence of charcoal rot disease. Observations were carried out during the critical growth stages, specifically the flowering and pod-setting phases. On July 20, approximately 10–15% of mature sesame plants in both fields exhibited symptoms indicative of the disease, including wilting and stunted growth (Figure 1a).

As the infection advanced, progressive symptoms such as premature leaf defoliation became evident, ultimately leading to plant mortality. Affected plants displayed characteristic blackened stems, a hallmark of charcoal rot infection (Figure 1b).

Upon closer inspection, recently shed leaves were found around symptomatic plants, marking the initial stage of disease development. Additionally, in later stages, dried leaves and pods remained attached to the darkened, deteriorating stems of infected sesame plants, further confirming the severity of the outbreak (Figure 1c).

3.2. Colony growth morphology characterization

Through the isolation procedure, a total of seven distinct isolates of *M. phaseolina* were successfully recovered from infected plants observed in the field. Initially, the colonies of these isolates exhibited a light pigmentation, which gradually darkened over time, eventually turning black (Figure 2).

Figure 1. Wilting and stunted growth (a), premature leaf defoliation and the death of plant (b), and severe infections that make the stem darkened (c) due to *M. phaseolina* infections

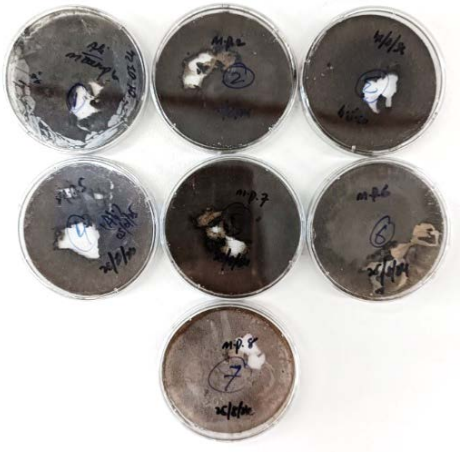

Figure 2. Colony growth color and pattern (8 day-old) of *M. phaseolina* isolates on PDA plates.

Microscopic examination revealed that the hyphae were predominantly brown to black in color, septate, and displayed extensive branching patterns. The microsclerotia of these isolates were initially brown but later transitioned to a darker brown or black hue. Their shape was generally irregular; however, they frequently appeared spheroid or oblong in structure. The size of the microsclerotia was recorded to be approximately 100 × 500 µm in diameter (Figure 3). These morphological characteristics align with previous descriptions of *M. phaseolina* reported in the literature, reaffirming their consistency with established diagnostic features.

3.3. Pathogenicity confirmation

The germination of sesame seeds was observed and recorded. Successful pathogen establishment was evident in the infected/treated soil. The treated soil facilitated infection in newly emerging seedlings at the initial growth stage (Figure 4). Over time, within 30–35 days, the infected plants exhibited severe wilting, roots blackening with infection and ultimately died, in contrast to the untreated plants, which remained healthy. Reconfirmation of the pathogen was conducted by reisolating it from the roots of infected seedlings and culturing it on PDA plates.

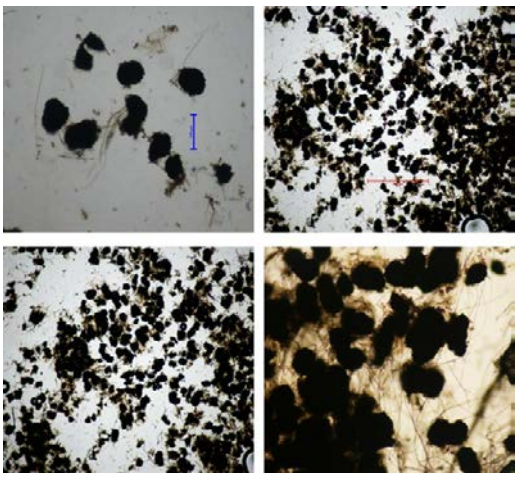

Figure 3. The micromorphological characteristics of the collected *M. phaseolina* isolates, including the development of microsclerotia and mycelial growth, were observed and analyzed under a 10× magnification lens.

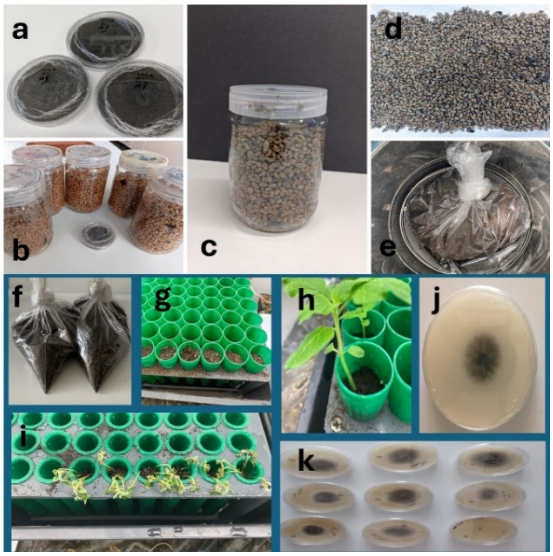

Figure 4. a) pure culture of *M. Phaseolina* used for the prepration of spore suspension, b) prepared suspension was added in each wheat filled bottle and placed in incubator at 30C, c) *M. phaseolina* grows on the wheat seeds/growth development in jar, d) two-three night over dryed the wheat seed in full controlled condition in the incubator with carefully covering of autoclaved filler papers, e) peat moss soil was autoclaved before mixing the fungus developed wheat seeds, f) after autoclaving the wheat seeds mixed with the soil and store for one week in growth chamber, g) inoculated soil filled in the growth tubes and 3-5 seeds of sesame were sown in each tubes, h) control plant/non-inoculated plant healthy sesame plant, i) inoculated plant showing the blacken of rhizospheric stem and died after the 30-35 days, j-k) reisolation of *M. phaseolina* fungus from the root of the died plants.

3.4. Molecular characterization and confirmation of *M. phaseolina* using the specific PCR primers

Polymerase Chain Reaction (PCR) successfully amplified *M. phaseolina* DNA in all seven symptomatic field isolates. The species-specific primers generated distinct PCR amplicons of approximately 350 bp, consistent with the expected fragment size as outlined in the methodology of Babu et al. (2007). The PCR product profiles are presented in Figure 5, confirming the presence of *M. phaseolina* in the tested samples. In contrast, no amplification bands were observed in the negative control (water), reinforcing the specificity of the assay and the absence of contamination in the experimental setup.

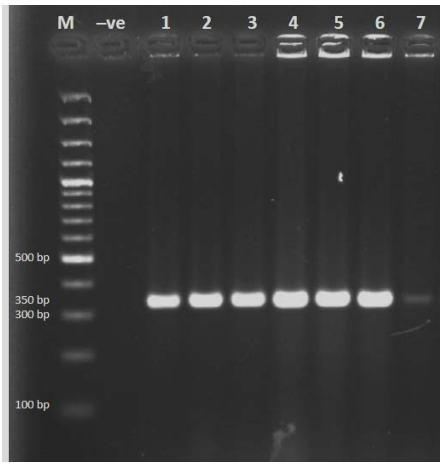

Figure 5. PCR amplification results of *M. phaseolina* isolates using the specific primers MpKRI/MpKFI. Lane M represents a 100 base pair molecular weight marker, while Lane -ve serves as the negative control. Lanes 1 to 7 correspond to the DNA extracted from collected and isolated samples, all of which exhibit the expected 350 bp amplicon, confirming the presence of *M. phaseolina*

4. Discussion

The prevalence of fungal plant pathogens has become a major concern and threat in imported agricultural crops, particularly in sesame. Sesame is an important oilseed crop. The production of sesame has declined due to the fungal pathogen *M. phaseolina* a reason behind the charcoal root rot disease of sesame. During the present study, morphological and molecular confirmation was done using the specific primers of *M. phaseolina*. The morphological characterization confirmed that the wilting of the whole plant and the blackening of the stem near the soil surface are infections of *M. phaseolina*. Microscopic characterization and colony growth showing the same pattern result in already reported studies (Akhtar et al., 2011; Chowdhury et al., 2014; Adhikary et al., 2019). Iqbal and Mukhtar (2014) reported significant morphological variations among different isolates of *M. phaseolina* collected from multiple host plants. These variations were evident in growth patterns, pigmentation, and the production of pycnidia, indicating substantial diversity within the species. Likewise, Beas-Fernández et al. (2006) documented morphological and pathogenic differences among *M. phaseolina* isolates obtained from diverse host plants and even from distinct tissues of the same host. Their findings suggest that environmental factors and host specificity may influence the phenotypic and pathogenic characteristics of this fungal pathogen.

Molecular markers are advanced techniques to detect the presence of pathogens in plants. It's an effortless and accurate approach to confirm the pathogen in an exact way. The internal transcribed spacer (ITS) regions of ribosomal RNA (rRNA) genes possess unique characteristics that facilitate the precise identification of fungal pathogens (Mahmoud and Zaher, 2015). The sequencing of the 18S rRNA gene within the ITS region has been widely employed for the molecular identification of *M. phaseolina* across various host plants (Babu et al., 2007; Romanelli et al., 2014; Khan et al., 2017). In this study, a more specific set of primers, MpKF1 and MpKR1, designed from conserved ITS sequences, successfully amplified a distinct 350 bp fragment. The high specificity of these primers ensured accurate differentiation of *M. phaseolina* from other soilborne fungal pathogens. Given that this 350 bp amplicon was absent in non-target fungal species, it can serve as a reliable molecular marker for species-specific detection of *M. phaseolina*, making it a valuable tool for plant disease diagnostics and pathogen surveillance (Babu et al., 2007). During the present study, *M. phaseolina* specific markers used to confirm the pathogen behind the charcoal rot of sesame. These specific markers are previously used in seveal studies to accurate confirmation of the *M. phaseolina* pathogen (Babu et al., 2007; Yousef, 2021).

5. Conclusion

Despite the extensive use of fertilizers and chemical treatments, the incidence of charcoal rot disease remains a significant challenge and poses a serious economic threat to many vital crops. This enduring issue highlights the need for a detailed molecular investigation of *M. phaseolina*. By analyzing its genetic diversity and key biological characteristics, researchers can gain deeper insights into its population structure and variability. Such information is crucial for developing effective disease management strategies, ultimately enhancing sesame yield and supporting breeding programs aimed at producing more resilient sesame cultivars.

Conflict of interest

The authors declare no conflicts of interest.

Authorship contribution statement

A. A.: Conceptualization & Study Design, Field Surveys & Sample Collection, Laboratory Work & Data Analysis,Molecular Characterization & PCR Analysis, Manuscript Drafting & Writing. M. T.: Field Surveys & Sample Collection, Laboratory Work & Data Analysis, Manuscript Drafting & Writing. M. A. N.: Critical Review & Editing. F. Ö.: Critical Review & Editing. F. S. B.: Critical Review & Editing, Supervision & Project Administration.

References

Adhikary, N.K., Chowdhury, M.R., Begum, T. & Mallick, R. (2019). Integrated management of stem and root rot of sesame (*Sesamum indicum* L.) caused by *Macrophomina phaseolina* (Tassi) Goid. International Journal of Current Microbiology & Applied Sciences, 8(4), 22-24.

Akhtar, K.P., Sarwar, G. & Arshad, H.M.I. (2011). Temperature response, pathogenicity, seed infection and mutant evaluation against *Macrophomina phaseolina* causing charcoal rot disease of sesame. Archives of Phytopathology and Plant Protection, 44(4), 320-330.

Babu, B.K., Saxena, A.K., Srivastava, A.K. & Arora, D.K. (2007). Identification and detection of *Macrophomina phaseolina* by using species-specific oligonucleotide primers and probe. Mycologia, 99(6), 797-803.

Balabaskar, P., Renganathan, P. & Sanjeevkumar, K. (2015). Occurrence of sesame root rot disease in Cuddalore district of Tamil Nadu and analysis of the variability in cultural characteristics and pathogenicity among isolates of *Macrophomina phaseolina* (Tassi) Goid. European Journal of Biotechnology and Bioscience, 3(8), 55-59.

Beas-Fernández, R., De Santiago-de, S.A., Hernández, D.S. & Mayek-Pérez, N. (2006). Characterization of Mexican and non-Mexican isolates of *Macrophomina phaseolina* based on morphological characteristics, pathogenicity on bean seeds and endoglucanase genes. Journal of Plant Pathology, 88, 53-60.

Chowdhury, S., Basu, A., Ray Chaudhuri, T. & Kundu, S. (2014). In-vitro characterization of the behaviour of *Macrophomina phaseolina* (Tassi) Goid at the rhizosphere and during early infection of roots of resistant and susceptible varieties of sesame. European Journal of Plant Pathology, 138, 361-375.

Gupta, R.N., Saharan, H.S., Rathi, A.S., Avtar, R. & Mandhania, S. (2020). Role of non-conventional chemicals in biochemical defence against charcoal rot of sesame incited by *Macrophomina phaseolina* (Tassi) Goid. International Journal of Current Science, 8(2), 656-660.

Iqbal, U. & Mukhtar, T. (2014). Morphological and pathogenic variability among *Macrophomina phaseolina* isolates associated with mungbean Wilczek from Pakistan. The Scientific World Journal, 1-9.

Khan, A.N., Shair, F., Malik, K., Hayat, Z., Khan, M.A., Hafeez, F.Y. & Hassan, M.N. (2017). Molecular identification and genetic characterization of *Macrophomina phaseolina* strains causing pathogenicity on sunflower and chickpea. Frontiers in Microbiology, 8, 1309.

Mahdy, E.E., El-Shimy, A.A., Sayed, M.A., Mahmoud, A.F. & Salah, A. (2023). Pedigree selection in improving seed yield and tolerance of sesame to *Macrophomina phaseolina* (Tassi) Goid. Egyptian Journal of Agronomy, 45(1), 31-44.

Mahmoud, S.A., El-Sharkawy, E.S. & Emam, M.A. (2024). Breeding sesame for resistance to charcoal rot caused by *Macrophomina phaseolina*. SVU-International Journal of Agricultural Sciences, 6(2), 18-35.

Nayar, N.M. & Mehra, K.L. (1970). Sesame: Its uses, botany, cytogenetics, and origin. Economic Botany, 24, 20-31.

Romanelli, A.M., Fu, J., Herrera, M.L. & Wickes, B.L. (2014). A universal DNA extraction and PCR amplification method for fungal rDNA sequence-based identification. Mycoses, 57, 612-622.

Sarkar, T.S., Biswas, P., Ghosh, S.K. & Ghosh, S. (2014). Nitric oxide production by necrotrophic pathogen *Macrophomina phaseolina* and the host plant in charcoal rot disease of jute: complexity of the interplay between necrotroph–host plant interactions. PLoS One, 9(9), e107348.

Yilmaz, M. (2022). Determination of yield and yield components of some sesame (*Sesamum indicum* L.) genotypes in the eastern Mediterranean transitional conditions. Ege Üniversitesi Ziraat Fakültesi Dergisi, 59, 709-715.

Yousef, H. (2021). Pathogenic variation and molecular characterization of *Macrophomina phaseolina*, the cause of sesame charcoal rot. Egyptian Journal of Phytopathology, 49(1), 151-165.