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ISOLATION, CHARACTERIZATION AND PATHOGENICITY ASSESSMENT OF *Macrophomina phaseolina* ASSOCIATED WITH CHARCOAL ROT OF SESAME

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ABSTRACT

The present investigation was undertaken to isolate, purify, identify, and confirm the pathogenicity of the causal organism associated with charcoal rot of sesame. Diseased stem and root samples exhibiting typical symptoms were collected from the Instructional Farm, College of Agriculture, Jodhpur. The pathogen was isolated and purified using the hyphal tip culture technique. The fungus initially produced whitish, dense mycelial growth, which gradually turned brown to black with age, along with the formation of numerous black, hard sclerotia within 10–15 days. Based on cultural and morphological characteristics, including septate hyphae and spherical to oblong sclerotia (38.40–42.82 µm), the pathogen was identified as *Macrophomina phaseolina*. Pathogenicity was confirmed under pot conditions by fulfilling Koch's postulates using seed, soil and seed-cum-soil inoculation techniques. The results revealed that the highest disease incidence (71.79%) was recorded in the seed-cum-soil inoculation method, followed by soil inoculation (58.70%) and seed inoculation (43.14%). Correspondingly, minimum seed germination (65.00%) was observed in seed-cum-soil inoculation, while maximum germination (93.33%) was recorded in the uninoculated control. Inoculated plants exhibited typical symptoms such as wilting, chlorosis, root and stem rot, and formation of black sclerotia. The study conclusively establishes *M. phaseolina* as the causal agent of charcoal rot in sesame and identifies seed-cum-soil inoculation as the most effective method for disease establishment. These findings provide a basis for further research on disease management and pathogen variability under field conditions.

Key words : *Macrophomina phaseolina*, *Sesamum indicum*, Hyphal tip culture, Pathogenicity, Sclerotia, Inoculation techniques.

Introduction

Sesame (*Sesamum indicum* L.), belonging to the family Pedaliaceae, is one of the oldest cultivated oilseed crops and is widely grown in tropical and subtropical regions (Weiss, 1971; Sharma *et al*., 2023). It is valued for its high oil content, nutritional richness, and remarkable oxidative stability, earning it the title "Queen of Oilseeds" (Bedigian and Harlan, 1986). It possesses approximately 50% oil, 23% protein and 15% carbohydrates, making it a preferred crop over diverse agro-climatic regions varied from semi-arid tropics to temperate regions (Wei *et al*., 2022). Despite its economic and nutritional importance, sesame productivity is considerably constrained by several diseases *viz*., charcoal rot, leaf blight, powdery mildew, stem blight, leaf spot, wilt, bacterial leaf spot and phyllody. Among them charcoal rot is one of the most destructive diseases causing significant yield losses (Karibasappa *et al*., 2020). Charcoal rot, caused by *Macrophomina phaseolina* (Tassi) Goid (syn. *Rhizoctonia bataticola*), is a soil-borne fungal disease that poses a serious threat to sesame cultivation, particularly under high temperature and moisture stress conditions (Choudhary *et al*., 2022). Sesame charcoal rot symptoms included seedling blight, root rot, stem

discolouration, vascular tissue shredding and the development of many black microsclerotia on infected tissues, which eventually led to premature drying and plant mortality (Singh *et al*., 2014; Maiti *et al*., 2015). Yield losses caused by disease were reported to be considerable, particularly in dry and semi-arid locations, where environmental circumstances promoted pathogen multiplication (Gupta *et al*., 2012).

M. phaseolina is a necrotrophic fungus with a wide host range, infecting more than 500 plant species across diverse agro-ecological regions (Mihail and Taylor, 1995; Yadav *et al*., 2025). It survives in soil and infected plant debris primarily in the form of microsclerotia, which can persist for several years and serve as the primary source of inoculum. Morphologically, the fungus produces hyaline, septate hyphae that later turn brown with age, with characteristic right-angled branching and constrictions at the point of origin. Microsclerotia are spherical to oblong, initially light brown and gradually turning dark black. Under certain conditions, the pathogen may also produce pycnidia containing hyaline, unicellular conidia, although this stage is rarely observed in nature (Lakhran and Ahir, 2020).

Several researchers conducted substantial research on the isolation and characterisation of *M. phaseolina* to better understand its variability and pathogenic potential. The fungus was commonly isolated on Potato dextrose agar (PDA) using normal tissue isolation techniques, where it grew rapidly with white to grey mycelium that gradually darkened due to the production of microsclerotia (Dhingra and Sinclair, 1978). Morphological characterisation comprised studies of colony morphology, growth rate, hyphal structure, and the size, shape and distribution of microsclerotia. However, significant heterogeneity in cultural and physical features between isolates was discovered, demonstrating genetic diversity within the pathogen population (Su *et al*., 2001; Gupta *et al*., 2012). Pathogenicity testing is considered a vital step in determining the pathogen's causative involvement. Several approaches, including soil inoculation, stem inoculation, and seedling assays, have been used to assess the virulence of various isolates (Dhingra and Sinclair, 1978; Mayek-Pérez *et al*., 2002). Fulfillment of Koch's postulates via artificial inoculation and symptom reproduction remained critical for determining the pathogenicity of the isolates. The variability in virulence across isolates was also mentioned, underlining the need for comprehensive pathogenicity assessment (Kaur *et al*., 2012; Gupta *et al*., 2012).

Given the economic importance of sesame and the devastating nature of charcoal rot, creating effective disease control measures required a thorough understanding of the pathogen through isolation, morphological and cultural characterization, and pathogenicity testing. As a result, the current study was conducted to isolate, describe, and assess the pathogenicity of *M. phaseolina* associated with sesame charcoal rot, with the goal of establishing baseline data for future research and integrated disease management measures.

Materials and Methods

Diseased sesame plants exhibiting typical symptoms of charcoal rot were collected from the Instructional Farm, College of Agriculture, Jodhpur, and infected stem and root portions were used for isolation of the causal organism. The samples were thoroughly washed under running tap water to remove adhering soil particles, and small tissue bits (approximately 5 mm) were excised from infected portions. These tissues were surface sterilized with 0.1% sodium hypochlorite solution for 1 minute, followed by three successive rinses with sterile distilled water, and then blotted dry on sterile filter paper (Dhingra and Sinclair, 1995). The sterilized tissue pieces were aseptically transferred onto PDA slants under laminar airflow conditions and incubated at 28±2°C for 7 days in a BOD incubator (Booth, 1971). To obtain a pure culture, the single hyphal tip isolation technique as described by Rangaswami and Mahadevan (2004) was employed, wherein a suspension containing 5–6 hyphal fragments per microscopic field (10×) was prepared, and 1 ml of the suspension was spread uniformly on 2% plain agar plates. After incubation at 28±2°C for 24 hours, a single hyphal tip was identified under a microscope (10×), excised using a sterile needle and transferred onto PDA slants, which were further incubated at 28±2°C. The purified culture was maintained by periodic sub-culturing on PDA slants and stored at 4°C for further studies (Subramanian, 1971).

PDA medium was used for isolation and culturing of the pathogen, and its pH was adjusted to 6.8 using 1N NaOH or 1N HCl before sterilization at 121°C (1.1 kg/cm² pressure) for 20 minutes (Aneja, 2003). The medium was prepared by boiling 200 g of peeled potato slices in 500 ml distilled water and filtering through muslin cloth, while 20 g dextrose and 20 g agar-agar were dissolved separately in 500 ml distilled water; both solutions were then mixed, and the final volume was adjusted to 1 litre prior to sterilization (Panda *et al*., 2017). The sterilized molten medium was poured aseptically into sterile Petri plates (90 mm diameter) at 15–20 ml per plate under laminar airflow conditions and allowed to solidify (Sahu

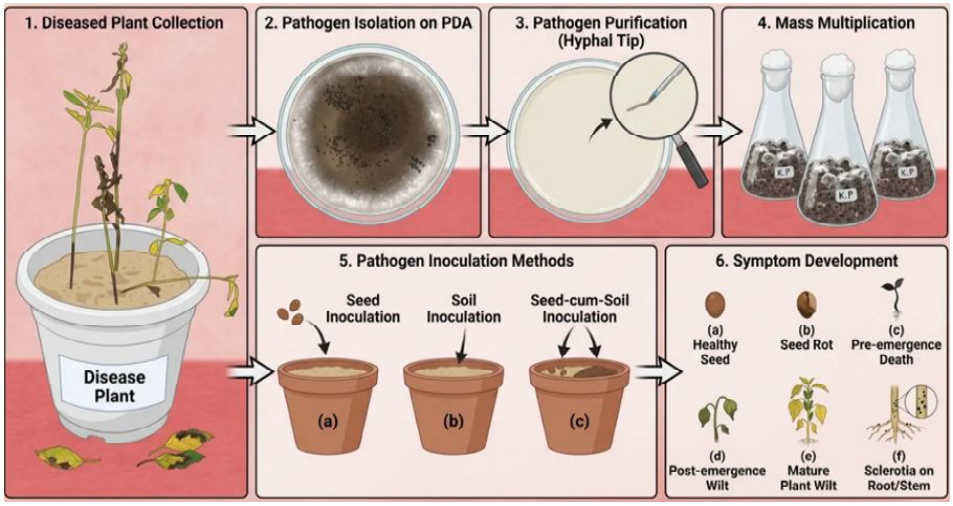


Fig. 1 : Isolation, purification and pathogenicity of *M. phaseolina* in sesame.

et al., 2018). For slant preparation, approximately 8–10 ml of PDA medium was dispensed into culture tubes, plugged with cotton, sterilized and allowed to solidify in a slanting position, after which the slants were stored at 4°C. The pathogen culture was maintained by regular sub-culturing on PDA plates and incubated at 28±2°C for 7 days, and cultures were preserved at 4°C with sub-culturing at 20–25 day intervals to maintain viability (Subramanian, 1971).

The pathogen was identified based on cultural and morphological characteristics, wherein the colony initially appeared white to grey and gradually turned black with age, producing black, spherical to oblong, and irregular microsclerotia. Microscopic observations revealed septate hyphae along with characteristic sclerotia, and slides prepared from 10-day-old cultures were examined under a microscope and compared with standard descriptions available in literature (Barnett and Hunter, 1998; Lakhran and Ahir, 2020). For microscopic examination, a small portion of fungal growth was transferred onto a clean sterile slide using a sterile needle, stained with 0.1% lactophenol cotton blue, and observed under a microscope for detailed morphological characterization (Dhingra and Sinclair, 1995). Mass multiplication of the pathogen, *M. phaseolina*, was carried out using sorghum grains following the method of Kataria and Grover (1976). Sorghum grains were soaked overnight, drained and 100 g grains along with 20 ml water were filled into 250 ml flasks, which were then sterilized at 121°C for 20 minutes (Aneja, 2003). After cooling, the grains were inoculated with 2–3 mycelial discs (5 mm diameter) taken from a 7-day-old fungal culture and incubated at 28±2°C for 20 days. The fully colonized grains were used as inoculum and mixed thoroughly with sterilized soil at 5% (w/w) for further experimentation (Kataria and Grover, 1976).

The pathogenicity of the isolated fungus was confirmed by following Koch's postulates (Koch, 1890) through seed, soil, and seed-cum-soil inoculation methods (Fig. 1). Sterilized soil was filled in earthen pots of 30 cm diameter, and inoculum was applied at the rate of 20 g per pot, followed by moistening and incubation for 72 hours prior to sowing (Nene and Thapliyal, 1993). Surface-sterilized seeds of sesame variety RT-351 were sown at the rate of 10 seeds per pot with three replications, while pots without inoculum served as control. Observations on disease development were recorded at 45 days after sowing (DAS) and the pathogen was re-isolated from infected plants and compared with the original culture to confirm pathogenicity (Agrios, 2005). In the seed inoculation method, surface-sterilized seeds were rolled over a 7-day-old fungal culture grown on PDA and then sown in pots, whereas uninoculated seeds served as control (Nene and Thapliyal, 1993). In the soil inoculation method, sterilized soil mixed with farmyard manure in a 3:1 ratio was inoculated with fungal culture at 20 g per pot, and surface-sterilized seeds were sown, while uninoculated soil served as control (Dhingra and Sinclair, 1995). In the seed-cum-soil inoculation method, both seed and soil were inoculated simultaneously, wherein inoculated seeds were sown in inoculated soil, and uninoculated seeds in sterile soil served as control (Agrios, 2005).

Results and Discussion

Charcoal rot caused by *M. phaseolina* is regarded as one of the most devastating and widespread diseases affecting sesame, especially under conditions of high temperature and moisture stress (Dhingra and Sinclair, 1978; Su *et al*., 2001; Gupta *et al*., 2012). The present study revealed successful isolation, identification, pathogenicity confirmation, and overall characterization

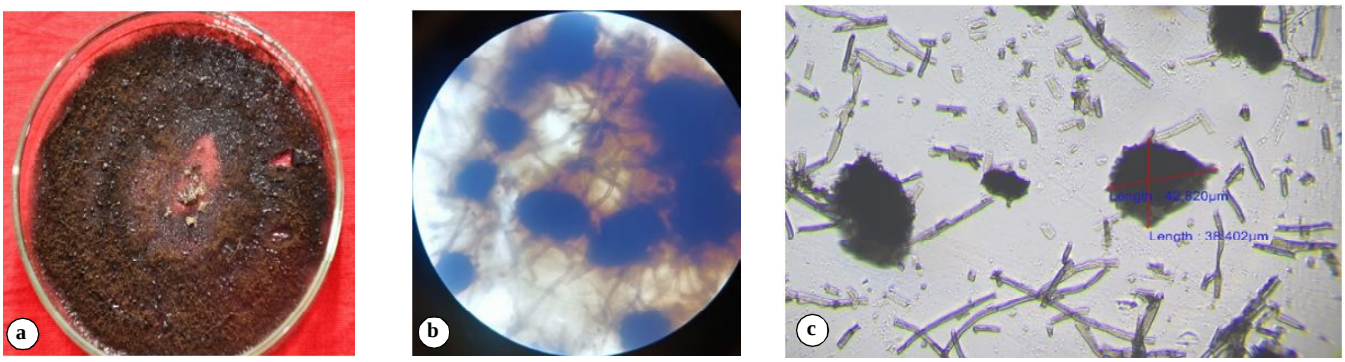


Fig. 2 : a) Sclerotia formation on PDA; **b)** Microscopic view of sclerotia; **c)** Measurement of sclerotia.

Table 1 : Standardization of inoculation techniques for charcoal rot of sesame.

Inoculation technique	Seed germination (%)*	Per cent disease incidence
Soil inoculation	76.67 (50.06)	58.70
Seed inoculation	85.00 (58.21)	43.14
Seed + Soil inoculation	65.00 (40.54)	71.79
Uninoculated control	93.33 (68.96)	0.00
SEm ±	1.073	1.458
CD (p = 0.05)	3.425	4.487

*Average of six replications.
Figures in parentheses are angular transformed values.

of the charcoal rot pathogen affecting sesame. Diseased plants exhibiting typical symptoms were collected from the Instructional Farm, College of Agriculture, Jodhpur, and infected stem and root tissues were subjected to surface sterilization and plated on PDA medium under aseptic conditions. Within seven days of incubation at 28±1°C, profuse fungal growth was observed, which initially appeared as whitish mycelium and gradually turned brownish-black with age. After 10–15 days, numerous black, hard sclerotia developed, particularly at the peripheral regions of the colony (Fig. 2a). Similar rapid growth and sclerotial development of the pathogen were also reported by Mehta (1951) and Pandey *et al*. (2020), indicating the typical cultural behavior of *M. phaseolina*. The fungus was purified using the hyphal tip culture technique to obtain a pure culture, which exhibited uniform colony growth with abundant aerial mycelium and embedded sclerotia on the agar surface. The morphological characterization revealed that the fungus produced hyaline, septate hyphae during early growth stages, which later became brownish-black with pronounced septation. Secondary hyphae showed characteristic right-angled branching, and sclerotia were observed to be round, irregular to oblong in shape, measuring between 38.40 to 42.82 µm (Fig. 2c). Initially light brown, the sclerotia turned dark brown to black upon maturity and the presence of pycnidial structures further supported identification. Based on these cultural and morphological characteristics, the pathogen was identified as *M. phaseolina* (the pycnidial stage of *R. bataticola*), which is in close agreement with earlier findings of Lakhran and Ahir (2020) and Barnett and Hunter (1998).

Pathogenicity of the isolated fungus was confirmed through pot culture experiments by fulfilling Koch's postulates using seed, soil, and seed-cum-soil inoculation methods. The results indicated that the pathogen caused seed rot, pre-emergence, and post-emergence mortality in sesame plants. Among the different inoculation techniques, the highest disease incidence (71.79%) was recorded in the seed-cum-soil inoculation method, followed by soil inoculation alone (58.70%), while the lowest germination (65.00%) was also observed under seed-cum-soil inoculation (Fig. 3; Table 1). In contrast, the highest seed germination (93.33%) was recorded in the uninoculated control, clearly indicating the detrimental effect of the pathogen on seedling emergence and vigor. These findings are in close conformity with the results of Priyanka *et al*. (2022), who also reported maximum disease incidence under combined inoculation methods.

The disease symptoms appeared within 30–40 days after sowing, beginning with sudden wilting of seedlings, followed by yellowing and senescence of leaves within 2–3 weeks (Fig. 4). Black sclerotia developed externally on stems and internally within root tissues, and progressive colonization of the root system led to vascular blockage, impairing water and nutrient uptake. Ultimately, root decay occurred, and infected plants were easily uprooted due to weakening at the collar region. Similar symptom progression and disease severity were reported by

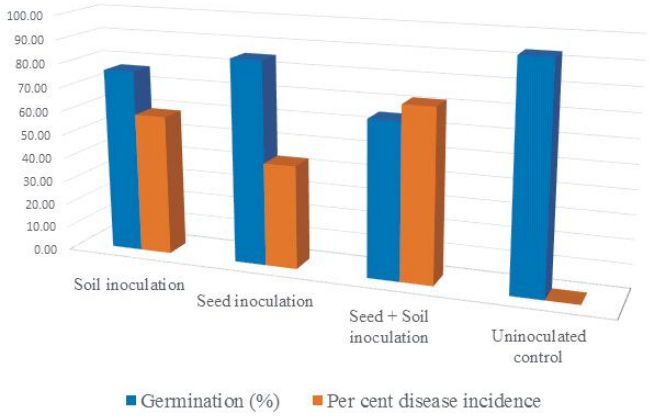


Fig. 3 : Standardization of inoculation techniques for charcoal rot of sesame.



Fig. 4 : Characteristic symptoms of charcoal rot of sesame on inoculated plant.

Lakhran and Ahir (2020), Aydogdu and Kurbetli (2024), El-Sayed *et al*. (2021), and Kumar *et al*. (2020), confirming the destructive nature of *M. phaseolina* under favorable conditions. The re-isolation of the pathogen from infected plant parts further confirmed its identity and pathogenicity, as the reisolated culture exhibited morphological characteristics identical to the original isolate.

Overall, the study clearly established that sesame, an important oilseed crop valued for its high-quality oil and antioxidant-rich compounds, is significantly affected by charcoal rot disease caused by *M. phaseolina*, particularly under high temperature and moisture stress conditions. The pathogen was successfully isolated, purified, and identified based on distinct morphological and cultural characteristics, and its pathogenicity was conclusively proven by fulfilling Koch's postulates. Among the inoculation methods tested, the seed-cum-soil inoculation technique proved to be the most effective in inducing disease, highlighting the importance of combined infection pathways in disease development. The findings of the present investigation not only confirm *M. phaseolina* as the causal agent of charcoal rot in sesame but also provide a reliable methodological approach for pathogenicity testing. Furthermore, the observed variation in disease incidence and its strong association with inoculation techniques emphasize the need for a deeper understanding of pathogen biology and infection mechanisms, which will be crucial for developing effective disease management strategies and for future studies on pathogen variability and host resistance.

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