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Macrophomina Phaseolina: An Emerging Disease

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Introduction

Macrophomina phaseolina is a fungal pathogen with more than 500 species of plant families. Canker occurs in many crops, which is called charcoal rot, because of the type of charcoal that is provided to the plant tissue. *M. phaseolina* is mainly found in soil in the environment and the host is different. In other words, it has the ability to infect monocotyledons and dicotyledons, causing uneven distribution in the soil (Mayek-Perez *et al*., 2001; Su *et al*., 2001). The pathogen is soil-bone and seed-borne, seed-to-seed from infected seeds (Pun et al., 1998). *Macrophomina* disease kills the plant before it emerges. Symptoms after emergence are the development of semicircular spindle shaped lesions with dark edges and light gray centers, sometimes covered with microsclerotia and sometimes pycnidia. Sclerotium can survive for long periods in soil (Baird *et al*., 2003). In addition to the plant area, several clinical reports have identified *M. phaseolina* as a human pathogen that can sometimes cause skin and other fungal infections (Tan *et al*., 2008; Srinivasan *et al*., 2009). The fungus has the ability to clear plant, animal and human infections in immuno-compromised patients receiving prophylactic antifungal therapy (Arora *et al*., 2011).

Taxonomy and nomenclature

- The genera *Macrophomina* was first developed by Petrak (1923) with the description of *Macrophomina philippinensis* from *Sesamum orientale* which was collected by G. M. Reyes in Philippines in 1921.
- However, the pycnidial stage of the fungus was named as *Macrophoma phaseolina* by Tassi (1901) and *M. phaseoli* by Maublanc (1905).
- Halsted (1890): The sclerotial state as *Rhizoctonia bataticola* (Taub.) Butler on sweet potato (*Ipomoea batatas*).
- Ashby (1927): He examined and compared the type specimens of the fungus from beans with other related genus and developed the binomial species i.e., *M. phaseoli* (Maubl.).

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- Goidanich (1947): He changed the binomial *M. phaseoli* to *M. phaseolina* (Tassi.) Goid., since the original specimen of *Macrophomina* which was collected by Tassi in 1901.
- Therefore, two names, *viz*., *M. phaseoli* (Maubl.) Ashby and *M. phaseolina* (Tassi.) Goid. became widely accepted in the literature.

Other synonyms for *M. phaseolina*

- *Sclerotium bataticola* (Taubenh, 1913)
- *Macrophoma cajani* P. Syd. and Butler (Sydow and Butler, 1916, Farr and Rossman 2010)
- *Macrophoma chorchori* (Sawada, 1916)
- *Macrophoma sesami* (Sewada, 1922)
- *Tiarosporella phaseolina* (Tassi; Aa, 1981).

✓ In India, the fungus was first isolated from cowpea (Shaw, 1912) and reported as *Sclerotium bataticola* by Sheikh and Ghaffar (1979).

✓ Van der Aa described the species as *Tiarosporella phaseolina* (Tassi.) Van der Aa in 1981.

✓ Currently, *M. phaseolina* (Tassi.) Goid. 1947 is officially recognized as the correct taxonomic name (CMI description of pathogenic fungi and bacteria no. 275) with sclerotial phase: *R. bataticola* (Holliday and Punithalingam, 1970).

✓ (Sutton (1980) reported that *Macrophomina* is a monotypic genus, composed of single species, i.e., "phaseolina."

Occurrence, distribution and economic importance

M. phaseolina infects more than 500 wild plant species, including several cash crops such as legumes and vegetables. It causes canker, wilt, charcoal rot, root dry rot, blight, leaf blight, stem blight and pre-emergence and post-emergence damping off (Singh et al., 1990). Root and stem rot of coniferous trees (McCain and Scharpf, 1989), fruit trees and various weeds (Songa and Hillocks, 1996). This fungus has a wide geographic distribution and is a serious problem in tropical and subarid to semiarid countries in Africa, Asia, Europe, North and South America (Gray *et al*., 1990; Abawi and Pastor-Corrales, 1990; Wrather *et al*., 2001).

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Table 1. First reports of occurrence of *M. phaseolina*

Sr. No.	Host plants	Country/continent	Diseases	References
1.	Guava	Varanasi, India	Wilt	Dwivedi (1990)
2.	Cassava	West Africa	Stem rot	Msikita *et al*. (1997)
3	Alfalfa and white clover	North America	Crown rot	Pratt *et al*. (1998)
4.	Coral hibiscus	India (Kerala)	Collar rot	Santhakumari *et al*. (2002)
5.	Safflower	Iran	Charcoal rot	Razavi and Pahlavani (2004)
6.	Soybean	Iowa	Charcoal rot	Yang and Navi (2005)
7.	Coleus	India	Root rot	Kamalakannan *et al*. (2006)
8.	Sunflower	Slovakia	Charcoal rot	Bokor (2007)
9.	Canola	Western Australia	Charcoal rot	Khangura and Aberra (2009)
10.	*Jatropha curcas*	India	Root rot	Sharma and Kumar (2009)
11.	Mungbean	China	Charcoal rot	Zhang *et al*. (2011)

Identification of fungus

The fungus colonies vary in colour from black to brown or gray depending on the culture, becoming darker with age. Petri dishes that produce large numbers of aerial hyphae and sclerotia are embedded in the hyphae and attached to the sediment or a smooth surface (Fig.1.). The hyphae are septate and are colored at first, but later turn honey-colored or black. Many brown to black sclerotia can be seen on the back of the petri dish. Vegetative mycelium is characterized by the formation of monilid or barrel-shaped cells and the formation of septum near the branches of the mycelium. Branches appear at right angles to the main pest, but

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branches also appear at acute angles (Dhingra and Sinclair, 1977). Microsclerotia are formed by groups of 50 to 200 individual cells and hyphae connected by melanin pigment.

Macrophomina microsclerotia are black in color and vary in size depending on the host and medium used (Short and Wyllie, 1978). Pycnidia are rarely produced in culture, but can be darkened in culture by alternating cycles of near-ultraviolet irradiation for 12 hours and darkness for 12 hours at 20 -24 °C. *M. phaseolina* usually produces circular or flat pycnidia varied from100 to 200 µm in diameter. The pycnidia are initially embedded in the host tissue and are dark or gray in colour, but as they mature, they turn black and emerge (Fig. 2). Pycnidia are membranous to subcarbonaceous with simple or definite truncate ostiole and conidiophores are solitary to colonial and simple rod-shaped, 10–15 µm long, bearing single-celled conidia. The central pores are circular and surrounded by thick-walled dark brown cells. The distributions produced in culture are dark brown to black, approximately 300 µm in diameter, and small to lageniform, with several layers of cells. The inner layer is coloured and the outer layer of the cells is dark brown to black. Conidia are twin or branched, simple. Conidia are spherical in shape, spherical, finally rounded at the base with truncate ends, and measure approximately 5–10 × 14–30 µm (Punithalingam, 1982). The apex is usually round and covered with a thin membrane that is continuous with the apex, up to 16-24×5-9 µm in size. The outer layer of the conidial wall is abducted and flattened, forming an apex with a cap-like or funnel-shaped conidial appendage.

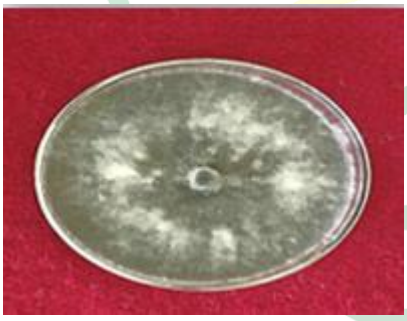

Fig. 1 Pure culture of *M. phaseolina*

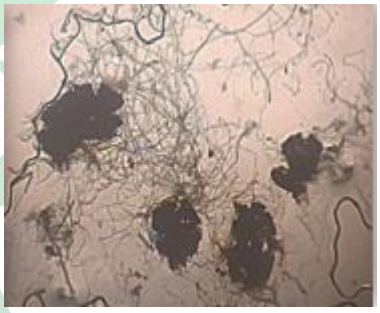

Fig. 2 microsclerotia with mycelium

Symptomatology

Spindle-shaped lesions with a light gray centre, brown edges and scattered pycnidial bodies are the first signs of *Macrophomina* disease on woody plants. In general, fungus can produce a variety of symptoms after a successful infection, from round-shaped lesions that are tied to the stem to long lesions that cause the plant to die. Deep, benign lesions extending to

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the hypocotyl and root surfaces have been observed in soybean (Ammon and Wyllie, 1972), chickpea (Singh and Mehrotra, 1982) and sorghum (Pedgaonkar and Mayee, 1990). When the lesions coalesce, a large mass appears on the branches or the whole plant, which quickly disappears and the plant dies (Dhingra and Sinclair, 1978). At the beginning of the development of the disease, these lesions are characterized by many black dots like pycnidia are evident on these lesions. Later, many pycnidia were found on most infected parts of the plant.

Epidemiology of *M. phaseolina*

Epidemiology is the simultaneous study of host populations and pathogens in their environment, known as the disease triangle. The presence of a susceptible host, a harmful pathogen, and environmental conditions that favour disease development make up the entire disease triangle. The disease cycle of *M. phaseolina* is shown in Figure 3. Microsclerotia produced in host root and stem tissue are the main source of inoculum and remain in the soil for 15 years (Short et al., 1980). Microsclerotia emerged from 0 to 20 cm soil depth, but are found in clusters on the soil surface (Alabouvette, 1990; Campbell and Van der Gaag, 1993) and are well adapted to survive adverse environmental conditions such as: Soil and temperatures with few nutrients. above 30°C, which is found in tropical and subtropical countries (Short *et al*., 1980).

Disease cycle of *M. phaseolina*

- Source of primary infection (Microsclerotia in soil, infected seeds and infected plant debris)
- Germination of microsclerotia (favourable temperature 28-35°C)
- Germ tube formation
- Development of appressoria
- Penetration of host epidermal cell walls (mechanical means, enzymatic digestion or via natural openings)
- Initially the hyphae grow intercellularly in the cortex and then through the xylem colonizing the vascular tissue
- Mechanical plugging of the xylem vessels by microsclerotia, toxins, enzymatic action and mechanical plugging during penetration
- Plant withers and dries up, break at the collar region
- Plant death

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- New disease cycle begins

Toxin production by *M. phaseolina*

- The phytotoxin was first identified from the culture filtrate of *M. phaseolina* (Siddiqui *et al*., 1979).
- The detailed structure of the phytotoxin which is an eremophilane sesquiterpenoid, specifically an epoxidized analogue of phomenone (Dhar *et al*., 1982). Kitahara *et al*. (1991) synthesized it semi-synthetically from (+)-sporogen-AO.
- The toxin affects the seed germination i.e., 50% even at a low concentration of 0.60–2.1 pg/g wet tissue (Bhattacharya *et al*., 1992).
- Bhattacharya *et al*. (1992) reported that enzyme-linked immunosorbent assay (ELISA) technique used for isolation and identification of the exotoxin secreted by *M. phaseolina* in the infected plant tissues.
- Mycotoxins produced by *M. phaseolina* include asperlin, isoasperlin, phomalactone, phaseolinic acid, phomenon and phaseolinone (Dhar *et al*., 1982; Mahato *et al*., 1987).
- Phaseolinone toxin was a non-host-specific heat-resistant exotoxin and inhibit germination of seed in black gram at concentration of 25 µg/ml (Bhattacharya, 1987). It also causes wilting in seedlings and necrotic lesions on leaves (Bilgrami et al., 1979).
- Mycotoxins played an important role either in the suppression of induced systemic resistance or in the activation of the plant defence response (Berestetskiy, 2008).
- UV-mutated non-toxigenic, avirulent mutants of *M. phaseolina* and a human and animal pathogen, *Aspergillus fumigatus* was reported to cause infection in black gram seedlings only in the presence of phaseolinone (Sett *et al*., 2000). This study confirmed phaseolinone as a major phytotoxic substance produced by *M. phaseolina* in the disease development.

Conclusion

The increasing incidence of plant diseases is a complex challenge and a serious economic threat to agricultural ecosystems. Despite the high use of chemicals and fertilizers, disease-related losses remain high. Characterizing pathogens in various ways, such as morphology and genetic structure, can play an important role in understanding population genetics and physiological parameters. Characterization based on morphological and cultural characteristics showed few differences between pathogenic and non-pathogenic fungal species.

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Therefore, rapid, simple and cost-effective methods are needed to identify, characterize, screen and control pathogenic or non-pathogenic populations.

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