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Review Article

Macrophomina phaseolina causal Organism of Charcoal Rot: Review Article

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Abstract: Chrcoal rot of Sorghum caused by *Macrophomina phaseolina* (Tassi) Goid is one of the more severe yield destabilizing factors causing serious yield losses each year. In recent years, *Macrophomina phaseolina* is becoming more prevalent in agricultural areas where climate change is leading to higher temperatures. We conducted a experiment in which, a series of fungal isolation, purification and identification from the infected plant parts. The plant show typical symptoms like Drooping of petioles and leaflets and in advance stage scattered sclerotial bodies. The infected isolates may be seen on the affected tissues after reinoculation and produced mycelia growth and sclerotia both plants and culture plates. On re-isolation it was found that the fungus was identical to the original isolate. It was observed that the infection was much higher in inoculated plants as compared to control.

Keywords: identification, *Macrophomina phaseolina*, pathogenecity, isolation, charcoal rot.

INTRODUCTION

Macrophomina phaseolina is a Botryosphaeriaceae plant pathogen fungus that causes damping off, seedling blight, collar rot, stem rot, charcoal rot, basal stem rot, and root rot on many plant species. *Macrophomina phaseolina* is a widespread, non-specific pathogen that can infect more than 500 hosts in about 100 families, including crops and weeds (Compendium of Sorghum Diseases. 2000; Compendium of Soybean Diseases. 1999). Economic crop hosts include cotton, groundnut, jute, maize, millet, potato, sesame, soybean and other beans, sunflower, sweet potato, tomato, and tobacco (Compendium of Sorghum Diseases. 2000). The fungus is a highly variable pathogen, differing in microsclerotial sizes and the presence or absence of pycnidia (Compendium of Soybean Diseases. 1999). Microsclerotia, 50-70 µm diameter (60-200 µm diameter when produced in laboratory), are black, smooth and round to oblong shape, uniformly reticulate, formed from hyphal agregates. Pycnidia, when present, are immersed in the host tissue and erumpent when mature. They are dark to grayish, globose, and membranous, with 100-200 µm in diameter and a truncate ostiole. Pycnidiospores are hyaline and single celled, ellipsoidal or ovoid, with a length-width ratio of 3:1, measuring 14-30 x 5-10 µm. Hyphal branches are formed in a right angle and may form a cross-wall at the constriction point, which can be confused with *Rhizoctonia* (C.C.D.1996; C.P.D.1997; C. S .D.2000; C.S. D. 1999).

The pathogen *Macrophomina phaseolina* of charcoal rot is a soil borne fungus often known by two distinct stags in its life cycle. First stage is pycnidial and second stage is sclerotial. Pycnidial stage is known as *Macrophomina phaseolina* (Tassi) goid (imperfect stage) and Sclerotial stage as *Rhizoctonia bataticola* (Taub) Bull. An interconnection between these two stages has been very well established by (Haigh, 1930) while Luttvell and Garenn (1952) cited the evidence that pycnidial state is capable of infecting and producing symptoms in host. The fungus can also thrive as saprophyte and become parasitic on living tissue of susceptible hosts.

The infection by *M phaseolina* was very severe during the hot and dry season and high wind accelerated heavy losses (Hiremath and Palakshappa, 1994) and the incidence was higher on hybrids than varieties (Narayan Rao *et al.*, 1997)

ISOLATION

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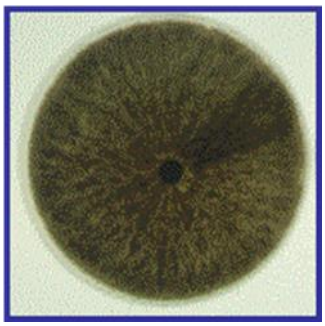
When microsclerotia are visible on host tissue isolation on culture media is easily accomplished using a dissecting scope. If microsclerotia are not evident, isolation should be attempted from the areas of the plant likely infected. Surface disinfestations of the tissue with 0.525% NaOCl solution for 1-3 minutes will enhance successful isolation. Standard solid culture media such as potato dextrose agar (PDA), cornmeal agar (CMA), lima bean agar (LBA), or water agar (WA) can be used and incubation at 28 to 35 C for 3-5 days will promote rapid growth of the fungus and exclusion of other microorganisms.

The microsclerotia density of *M. phaseolina* can be determined in soil using selective media (C. S .D.2000). A 5 g-subsample is first washed in 10% bleach and then rinsed over a 325 µm sieve. The subsample is then added to 100 ml of molten PDA amended with rifampicin (100 ml/L) and tergitol (1 ml/L) and distributed evenly over 10 plates. Colonies of *M. phaseolina* can be enumerated with an unaided eye by morphology after incubation at 28 C in darkness for 3 to 5 days.

IDENTIFICATION



Colony of *M. phaseolina* on PDA.

M.phaseolina (Tassi) goid. (syns. M. phaseolina (Maubl.)Ashby, Rhizoctonia bataticola (Taub.) Briton-Jones, Sclerotium bataticola Taub., and Botryodiplodia phaseoli (Maubl.) Thrium.), is a soilborne plant pathogen belonging to the phylum Deuteromycetes and class Coelomycetes. It is highly variable, with isolates differing in microsclerotial size and presence or absence of pycnidia. The pycnidial stage is not common on soybean, but is on peanut. Pycnida are initially immersed in host tissue, then erumpent at maturity. They are 100-200 µm in diameter; dark to grayish, becoming black with age; globose or flattened globose; membranous to subcarbonaceous with an inconspicuous or definite truncate ostiole. The pycnida bear simple, rod-shaped conidiophores, 10-15 µm long. Conidia (14-33 x 6-12 µm) are single celled, hyaline, and elliptic or oval.

Microsclerotia of M. phaseolina are jet black in color and appear smooth and round to oblong or irregular. Across isolates, microsclerotia vary on size and shape and on different substrates. Microsclerotia are formed from aggregates of hyphal cells joined by a melanin material with 50 to 200 individual cells composing an individual microsclerotia. Colony of M. phaseolina on PDA. Colonies in culture range in color from white to brown or gray and darken with age. Hyphal branches generally form at right angles to parent hyphae, but branching is also common at acute angles. Aerial mycelium with completely or partially appressed growth may or may not be produce in culture. Some isolates may form concentric growth rings.

SYMPTOMATOLOGY

Charcoal rot of Sorghum is a widespread root and stem disease caused by soil inhibiting fungus *Macrophomina phaseolina*. The term charcoal rot was first used by inhibiting Tatibenhaus (1913) in case of Sweet potato but in Sorghum it was first used by Dastur (1931). Since then based on the symptoms of the disease this terminology has been used by Plant Pathologists.

Although, the naturally as well as artificially inoculated Sorghum plants show symptoms due to other diseases also but the charcoal rot disease symptoms on the host plant are very prominent and distinct. Symptoms of the disease can be precisely recorded at three growth phases (Table 8)-

- Pre-emergence disease symptoms
- Post-emergence disease symptoms
- Symptoms on mature plant.

PRE-EMERGENCE DISEASE SYMPTOMS

The symptoms of charcoal rot vary and depends upon the time of infection. Seedlings can be infected when soil is dry and soil temperature is above 28°C for 2-3 weeks after planting. Infected seedlings exhibit circular to oblong, reddish brown lesions on emerging hypocotyl near the soil line. Lesions are not sunken. The reddish brown lesions of charcoal rot turn brown to nearly black after several days. Infected seedling may die if hot and dry conditions persist.

Although root infection can occur anytime during the season, the adventitious roots are formed in the soil but their number and size was greatly reduced. The roots turned brown and soon died. The fungus produced sclerotia, severely infected plants died and did not grow further.

The roots functioned almost normally. The plant matured although their conducting system was partially blocked and resulted in wilting at the later stage. Such roots showed extremely discolouration, darkening, sclerotia formation and the formation of water soaked lesions in some cases and matured plant may be less vigorous and may progressively become yellow, wilted and die.

Table-1. Pre_emergence and post emergence mortality of infected Sorghum plants at different ages.

S. No.	Treatments	Age of seedling/ plants in days	Replications R1	Replications R2	Replications R3	Replications R4	*Total Survival	%Survival
1.	Inoculum + Seed	10 20 30 60 90	4 4 3 3 3	4 4 3 2 2	3 3 2 3 3	5 3 2 2 2	16 14 10 10 10	80 70 50 50 90
2.	Check (Seeds only)	10 20 30 60 90	4 5 5 5 5	5 5 5 5 5	4 3 3 2 2	5 4 4 3 3	18 17 17 15 14	90 85 85 75 70

* Total of four replications (5 plants/replication)

Leaves remain attached to the petioles for several days after death. The diagnostic symptom of charcoal rot is easily observed after plant death. Numerous, minute, black specks ('pin head size') microsclerotia can be seen when the epidermal tissue is peeled away from the lower stems and root of affected plants. The microsclerotia are most abundant in the silvery to gray areas of the lower stems and taproots. Lower stems and taproots can be split open to reveal microsclerotia embedded within the cortical tissue as well as with in the pith tissues. Due to abundant microsclerotia, these tissues exhibit a 'grayish black coloration', (the diagnose feature of the disease charcoal rot).

(1)T-EMERGENCE DISEASE SYMPTOMS

The plant emerged out of the soil in 10 to 15 days. Severely infected plants gave a disease appearance i.e. their shoot formation was very weak. The leaves did not turn green but remained yellowish or yellowish brown (Richard *et al.,* 2000). The nodes and internodes were not distinguished and the plant hardly attained the IVth and Vth phase of growth (Mayee and Datar, 1986). Such plants did not show external growth of the pathogen on the plant bark, yet in some cases the fungal presence could be detected (Richard *et al.,* 2000). These plants died soon.

(3) SYMPTOMS ON MATURE PLANTS

However, mildly affected plants continued to grow up to 90 days period and started flowering and fruiting. In these plants, the disease was manifested at the soft and hard dough stage and the symptoms were quite typical of the charcoal rot disease. Initial symptoms develop on roots and appears as water soaked lesions. The lesions turns black with age. The fungus continues to invade plant tissue from the crown up and cause similar water soaking and discoloration in the pith. The pith eventually disintegrates leaving only the vascular strands in tact. As the disease progress the epidermal tissues may flake or shred off the stem and the black specks or microsclerotia may be seen in the epidermal and subepiderrnal tissues. Microsclerotia may be more prevelent at the stem nodes and give the stems the appearance of having been dusted.

In mildly infected plants the grain setting was comparatively better in comparison to the severely effected plants, where the no. and size of the earheads was reduced, and some viable grains were also produced . Infected leaves showed yellowing, drying and wilting.

The most characteristic outward symptom of the disease is lodging of infected plants. Other yield depleting factors associated with lodging are poor grain filling and premature ripening. The husk turned brown or dark brown at the base showing formation of sclerotia over the epidermal regions.

The grains in earhead showed poor setting and a few grains formed remained small in size. They were discoloured, shrunk and abortive. However, Injury from this disease does not usually become evident until the plant approaches maturity.

DISEASE CYCLE

The microsclerotia of *Macrophomina phaseolina* survive in the soil and crop debries for 1 to 3 years or more and are the primary source of inoculum for root infections. Microsclerotia are hard masses of fungal tissue that are resistant to adverse conditions. Infection results when the plant roots make contact with hyphal strands from germinating microsclerotia, usually near the soil surface, Microsclerotia are multi celled. A single microsclerotia may germinate repeatedly during the growing season, when conditions are favourable.

Hot and dry soil is favorable for disease development. The fungus first invades cortical tissues of the lateral roots. From there it continues to grow into

the taproot and uPward into the lower stem, finally producing microsclerotia.

External symptoms are usually not seen during the early stages of disease development. During later stages of disease development, microsclerotia are produced in diseased tissues. Yield losses from charcoal rot result poor earhead fill and reduced seed size. Seed may not carry the pathogen into the next year in the seed coat. After harvest, microsclerotia are protected in crop residue As residue breaks down, tnicrosclerotia are released into the soil.

Management

Cultural management methods must be implemented to minimize charcoal rot damage since there are no fungicides available for effective disease control.

Crop rotation out of a susceptible host is effective in some crop production systems. Rotation out of soybeans for three years may effectively reduce microsclerotia numbers and is useful for managing charcoal rot (Cloud, G. L., & Rupe, J. C. 1991). Corn is also a host for *M. phaseolina* but isolates appear to be specific to each crop (soybean and corn) though all isolates can infect both crops. Corn is not as good of a host to *M. phaseolina* as soybean so rotation with corn for three years may help reduce populations but not eliminate the pathogen from the soil. Rotation with a poor host such as cotton may only require one or two years to reduce inoculum levels in soil. For peanuts rotation with cotton or rice for two to three years may help reduce soilborne inoculum.

Early Planting will aid in earlier canopy closure that will help reduce soil temperatures and therefore reduce the competitive ability of *M. phaseolina*.

Avoid high plant populations. High plant populations can contribute to increase plant stress and competition for water increasing charcoal rot potential.

Fertility. Adequate levels of available P and K will reduce nutrient stress and encourage health plant growth.

Soil Moisture. Tillage practices which reduce soil moisture stress may reduce disease potential. Maintaining good soil moisture with irrigation from planting to pod fill may reduce disease potential.

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