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TECHNICAL DOCUMENT ON CHARCOAL ROT OF CUCURBITS

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Review Article

ABSTRACT

Charcoal rot of cucurbits is a serious disease spreading all around the world. Therefore, this technical document summarizes the current knowledge of cucurbits charcoal rot disease epidemiology, symptoms and signs, disease cycle, ecology and disease management strategies. Charcoal rot disease is caused by the pathogenic fungus *Macrophomina phaseolina*. The fungi belong to the family Botryosphaeriaceae, order Botryosphaeriales, class Dothideomycetes and phylum Ascomycota. *M. phaseolina* is characterized by the production of both pycnidia and sclerotia in host tissues and culture media. The disease symptoms on the plant represented by appearing spindle-shaped, water-soaked lesions on the stem, vine decline, wilt, and decline of the host plant resulting in plant death. Lesions dried out progressively, turned tan and cracked. So, affected plants decline and die before harvest. Sunken cankers also, appear on seedlings. The fruits also attacked by the fungus, especially, these which were in contact with the soil. Abundant black microsclerotia associated with the infection sites. These microsclerotia are distributed generally in clusters at the soil surface and are localized mainly at a depth of 0-20 cm. They can survive for 2-15 years depending on environmental conditions and presence of plant residues. So, they are considered as the main surviving propagules across different seasons. The plant root exudates induce germination of these microsclerotia and resulting in root infection. The charcoal rot disease can be managed by decreasing pathogen propagule in soil and host roots and avoiding favorable conditions for further pathogen survival and propagation. Agricultural methods (irrigation type, fertilization with organic amendments, tillage, etc.), grafted plants and solarization can affect charcoal rot disease. There are some chemical fungicides such as azoxystrobin, difenoconazole, carbendazim and benomyl can be used under laboratory and field conditions for disease controlling. Management strategies for controlling this disease also include using biocontrol agents such as *Bacillus* spp., *Streptomyces* spp., *Pseudomonas* spp., *Trichoderma* spp., *Gliocladium* spp. to prevent host infection or to suppress the growth of the pathogen and reducing the disease. All methods achieved a significant controlling of the disease and reduced the disease severity with different degrees.

Keywords: Charcoal rot; cucurbits; disease cycle; disease management; *Macrophomina phaseolina*.

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1. IMPORTANCE

Macrophomina phaseolina is a generalist soil-borne pathogenic fungus present all over the world, affecting at least 500 plant species in more than 100 families and causing significant yield losses (>60%). It causes diseases such as stem and root rot, charcoal rot and seedling blight [1,2].

Most reports of these disease are from agronomic crops such as corn, cotton, sorghum, and soybean [3]. However, charcoal rot remains an important disease of vegetable crops such as cucurbits [4]. Cohen et al. [2] and Cohen et al. [5] reported that *M. phaseolina* causes vine decline in cucurbits, which can be quite severe in melons under certain environmental conditions, and especially in the arid areas of the world [6]. Yield losses claimed by charcoal rot in Russia, Uruguay, Spain and United States were recorded to 25%, conversely under favorable conditions for the growth and development of the this soil-borne pathogen (100%) [6,7]. Khan [6] highlighted a high level of variation in physiology, morphology and pathogenesis of *M. phaseolina* isolated from different parts of the same plant.

2. SYMPTOMS AND SIGNS

Charcoal rot affects all cucurbits species. Generally, *Macrophomina* can cause a range of symptoms after a successful infection from restricted spindle-shaped lesions on the stem to extended lesions that result in the wilting of the plant. On seedlings, black, sunken cankers may appear on hypocotyls at the time of emergence. These cankers may develop a concentric ring pattern, stunt affected plants and cause wilt (Fig.1) [1,8,9].

When older plants are attacked, runners and crown leaves may turn yellow. Typically, a water-soaked lesion will occur at the soil level and extend several centimeters up the stem. Lesions dried out progressively, turned tan, and cracked. As the disease progresses, the stem of infected plants ooze amber-colored gum, and the stem eventually becomes dry and tan-to-brown in color. The stem may be girdled by the lesion, resulting in plant death. Numerous microsclerotia, visible as black specks, are embedded in the dead plant tissue. Affected plants decline and die before harvest (Fig.1) [1,10,11].

During the early stages of disease development, several black dots like pycnidia are evident on these lesions. Later on, several pycnidia can be observed on most of the infected parts of the plant [2,6].

This soil-borne fungus can attack fruit in contact with the soil. Brown, water-soaked lesions are symptomatic of fruit infection. Amber-colored droplets of exudates may form within the affected area. Eventually, the lesion dries up, turns light tan and microsclerotia form (Fig.1) [12,13].

3. CAUSAL AGENT AND DISEASE DEVELOPMENT

Macrophomina phaseolina (Tassi) Goidanich is a serious soil-borne pathogen affecting a wide range of cultivated and wild species, including those in the *Cucurbitaceae*, in warm temperate and tropical regions of the world. Disease incited by *M. phaseolina* is often referred to as charcoal rot because of the dark coloration of the parasitized host tissue [2,5,11-15]. *M. phaseolina* belongs to the family Botryosphaeriaceae, order Botryosphaeriales, class Dothideomycetes and phylum Ascomycota [16].

M. phaseolina is characterized by the production of both pycnidia and sclerotia in host tissues and culture media. The pycnidial state was initially named *Macrophoma phaseolina* by Tassi in 1901 and *Macrophoma phaseoli* by Maublanc in 1905. In 1927, Ashby maintained the name *Macrophomina phaseoli*, while Goidanich [17] proposed *Macrophomina phaseolina*. *Tiarosporella phaseolina* (Tassi) Van Der Aa was used in 1981 by Van Der Aa to designate the species [10,12].

Microsclerotia in soil, infected seeds or host tissues serve as primary inoculums source of *M. phaseolina*. They are distributed generally in clusters at the soil surface and are localized mainly at a depth of 0-20 cm [18]. This structure of resistance can survive for 2-15 years depending on environmental conditions, and whether or not the sclerotia are associated with host residues [8,12]. Factors that adversely affect the survival of these propagules include repeated freezing and thawing of soil, low carbon: nitrogen ratios in soil, and soil moisture content. Microsclerotia are formed from the aggregation of hyphae with 50 to 200 individual cells coupled by a melanin pigment. The microsclerotia of *Macrophomina* are black in color and their size varies from 50 to 150 µm according to the host and the media used (Fig.2) [10].

Root exudates induce germination of microsclerotia and root infection of hosts. It can infect the roots of the host plant at the seedling stage via multiple germinating hyphae. The infective hyphae enter into the plant through root epidermal cells or wounds. During the initial stages of pathogenesis, the mycelium penetrates the root epidermis and is restricted primarily to the intercellular spaces of the cortex of the primary roots. As a result, adjacent cells

collapse and heavily infected plants may die [12,19,20]. Once in the roots, the fungus affects the vascular system, disrupting the water and nutrient transport to the upper parts of the plants. At flower onset, the fungal hyphae grow intracellularly through the xylem and form microsclerotia that plug the vessels and disrupt host cells. Typical symptoms are yellowing and senescence of leaves that remain attached to the stems by the petioles, sloughing of cortical tissues from the lower stem and taproot, and the grey appearance of these tissues due to the abundance of microsclerotia that can result in a premature death of the host plant (Fig.2) [1,2].

After plant death, colonization by mycelia and formation of sclerotia in host tissue continue until tissues are dry. The mycelium and microsclerotia produced in infected plant material, including plant residues are the means of propagation of the pathogen. Microsclerotia in soil, host root and stems are the main surviving propagules. After decay of root and plant debris, microsclerotia are released into the soil (Fig.2) [18,21].

Environmental conditions like temperature, atmospheric humidity, and soil water potential play an important role in the viability and inoculum potential of *M. phaseolina* [6]. *M. phaseolina* is able to produce microsclerotia under relatively low water conditions; thus, survival of this inoculum is influenced by the soil matric water potentials. Viability of microsclerotia were drastically reduced at high water potentials (-30 J/Kg, field capacity), and was virtually not affected at low water potentials (-1.500 Kg/J, permanent wilting point) in a sandy loam soil [9,22].

Fig. 1. Symptoms of charcoal rot on stems and fruits of cucurbits

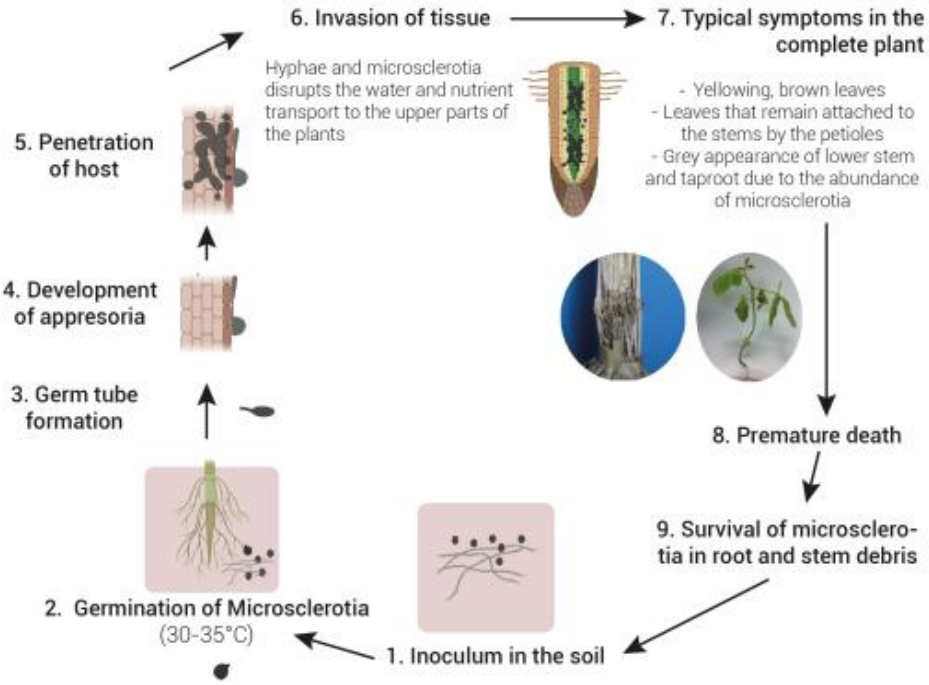

Fig. 2. Disease cycle of charcoal rot caused by *Macrophomina phaseolina*

Soil water content affects the gaseous conditions in the soil and may cause reduced microsclerotia survival by the reduction of O_2. Substances found in flooded soils such as alcohols, volatiles and increased levels of CO_2 can have a detrimental effect on the inoculum [22]. Microsclerotia germination is annulated in artificial atmospheres containing less than 16% of O_2 concentration in soil column systems; indicating that reduction in viability is not due to nutrient deprivation [9].

Goudarzi et al. [23] describe *M. phaseolina* fungal growth creating different matric and osmotic potentials by using polyethylene glycol (PEG 6000) and sodium chloride respectively, on *in vitro* conditions. Microsclerotia germination and radial growth increases as the osmotic and water potentials decreases. However, there is an optimum of - 0.6 MPa for the osmotic potential and - 1.2 MPa for the matric potential, this suggest that a positive turgor is maintained in the hypha of *M. phaseolina* during growth and this adaptation to survive in low water potentials is used for the pathogen to survive in host tissue under these conditions [9].

Sandy soils lead plant mortality of approximately 90% in comparison to loamy and clayey soils in which plant mortality reached 77% and 52%, respectively. In addition, in low levels of soil moisture disease severity is higher and the microsclerotia population in soil increases as well [24,25].

Microsclerotia are generally found in clusters on the soil surface and are well adapted to survive under adverse environmental conditions, such as low soil nutrient levels and temperature above 30°C which prevail in tropical and subtropical countries. The germination of microsclerotia occurs frequently in the temperature range of 28-35°C [2,10].

Repeated freezing and thawing of soil, low carbon to nitrogen ratios in soil, and soil moisture content are the most important factors that significantly affect microsclerotia survival [26]. Dhingra and Sinclair [27] and Olaya and Abawi [28] documented the enhanced production of microsclerotia under low water potentials that occurs during drought. High soil moisture has proved detrimental and reduced the survival of *M. phaseolina* sclerotia in soil [26].

4. DISEASE MANAGEMENT STRATEGIES

The primary aim of *M. phaseolina* management is to decrease pathogen propagule in soil and host roots, and avoid favorable conditions for further pathogen survival and propagation. Many control strategies have been evaluated in recent decades with varying degrees of success against this disease.

The chemical control of *M. phaseolina* is complicated, since there are no systemic fungicides that move towards the root. As far as we know, no fungicides have been registered to manage this soil-borne pathogen. Nevertheless, azoxystrobin,

difenoconazole, carbendazim, benomyl, dazome were evaluated under laboratory and field conditions against *M. phaseolina* at different concentration [5,29-32]. The results documented that the formation of sclerotia and mycelial growth are highly sensitive to carbendazim at 50 ppm [32]. Disease management combining cultural practices with chemicals have been reported, but no conclusive results could be drawn, requiring further investigations [5]. Although the effectiveness of some chemical fumigants has been confirmed [32,33], agro-environmental policies and the increasing negative perception of the public on the agrochemicals have led to the evaluation and comparison of chemicals agents with more sustainable alternatives to control charcoal rot disease on cucurbits [34-36].

Charcoal rot management involving the combination of grafted plants and fungicides application to the soil during the growing season has been recently documented by Cohen et al. [2] and Cohen et al. [5]. The results reported that the grafted melons did not wilt, compared to 80% wilting of the non-grafted melon. Moreover, chemical treatment of azoxystobin alone or in combination with chlorothalonil or mefenoxam drastically reduced the incidence of charcoal rot. Thus, grafting alone or in combination with chemical control can be used for disease management [2,5].

The impact of irrigation on the survival microsclerotia in soil and cucurbit roots has been studied by numerous researchers. Kendig et al. [37] noted that the irrigation has been one of the most effective means to manage charcoal rot for many plant species. The same authors showed that irrigation throughout the growing season reduces the colonization and population of *M. phaseolina* on the roots compared to non-irrigated plots, although the propagules remain during the season in both systems (irrigated and non-irrigated plots) and no symptoms were detected in the irrigated plots. Nischwitz et al. [38] revealed that irrigation at any time during the growing season of melon plants reduces the infection of charcoal rot disease. Furthermore, the irrigation type can also affect charcoal rot. The density of sclerotia in the soil and the number of diseased melon plants were higher in the drip-irrigated fields than in furrow-irrigated fields [38].

Tillage is a critical cultural measure that could affect the inoculum potential of *M. phaseolina*. If this pathogen requires a high inoculum density to infect cucurbits, then amplified dispersal on the soil profile could diminish the severity of charcoal rot. Conversely, if a low inoculum density is sufficient for infection, dispersal in this case may worsen the charcoal rot incidence and severity [39]. As low inoculums densities are sufficient to cause charcoal rot, tillage can augment the damage by *M. phaseolina*, especially when highly susceptible cucurbit species are cultivated, in which a soil sclerotial density < 1 microsclerotium per gram of soil can cause plant mortality of over 90% [40]. Tillage reduces the stratification of organic residue on the soil, which in turn can affect soil temperature and moisture [18], soil chemistry [41], soil animals population, and the microbial communities structure [42]. These changes in the physical, chemical and biological factors of the soil can in turn also affect the incidence and severity of charcoal rot.

Sheikh and Ghaffar [43] showed that an important method for reducing the viability of microsclerotia in the soil is the polyethylene mulching. Increasing soil temperatures to 52-65°C for 7 days in soil naturally infested with *M. phaseolina* reduced propagule viability with 100% (at a depth of 5 cm) and 50% (at a depth of 20 cm) reduction. Maintaining high soil moisture was necessary to increase soil thermal conduction in the mulched soil. Higher densities of bacteria and actinomycetes were found in heated soils compared to untreated soils [43]. The application of solar energy of moistened soil further increased this decline at the upper depths, nevertheless many propagules survived at the lower depths [43]. Amendments with nitrogen-enriched pearl millet residues significantly decreased the *M. phaseolina* population within 45 days by 94% [44]. The combined effects of irrigation, amendments and polyethylene mulching in soil naturally infested with *M. phaseolina* resulted in the almost complete eradication of the pathogen population with reduction ranged between 93 and 99% at a depth of 0-30 cm within 15 days. A considerable reduction (75-95%) was also obtained by natural heating of the irrigated soil for two weeks after fertilization with cruciferous residues [45]. Gamliel and Stapleton [46] and Gamliel and Stapleton [47] noted that the effect was mainly attributed to toxic volatiles (dimethyl sulphide, mercaptan, isothiocyanate, etc.) formed during the decomposition of cabbage residues. Biosolarization, which combines biofumigation and solarization, has been shown to be effective in reducing or stabilizing the population of *M. phaseolina* microsclerotia in soils [48]. The wide host range and high persistence of *M. phaseolina* microsclerotia make crop rotation, intercropping and lay period strategies less considered [49].

Management aimed to modify the soil environment, in favor of antagonistic organisms interfering with the pathogen, have also been attempted. Perez-Brandán et al. [50] pointed out that adoption of conservation

strategies such as direct seeding, revealed suppression of *M. phaseolina* microsclerotia promoted by higher microbial activity and abundance, with good development of healthy root systems. Similarly, Lodha et al. [45] noted that irrigation coupled with organic soil amendment, amplified the lytic bacteria population against *M. phaseolina*. Spagnoletti et al. [51] and Spagnoletti et al. [52] documented that the fertilization revealed different effects on the severity and intensity of charcoal rot disease; fertilization with phosphorus showed a decrease, whereas nitrogen increased the severity of the disease.

Management strategies to control charcoal rot also include the use of biocontrol agents to prevent host infection or to suppress the growth of the pathogen. Antagonistic microorganisms have been investigated for the control of charcoal rot on diverse species. Products made from bacteria (*Bacillus* spp., *Streptomyces* spp., *Pseudomonas* spp., etc.) and fungi (*Trichoderma* spp., *Gliocladium* spp., *Penicillium* spp., *Aspergillus* spp., etc.) are known to suppress *M. phaseolina* growth under specific conditions [53]. Such antagonistic activity is attributed to the production of hydrolases, including chitinases and glucanases, which degrade the main components of the fungal cell wall [54]. *Trichoderma* spp. are able to degrade fungal cell walls by the cellulase production [8]. In turn, *M. phaseolina* cell wall components stimulate the production of *T. harzianum* conidia. *T. harzianum* is able to decrease the severity of charcoal rot by 37-74% on melon plants grown in fields artificially infested with *M. phaseolina*. In the same way in commercial fields, melon plants from seeds treated with *T. harzianum* yielded 61% more fruit than plants from non-treated seeds in soils naturally infected with *M. phaseolina* [55]. *Trichoderma* spp. reduce the viability and longevity of *M. phaseolina* microsclerotia on decaying plant tissues [8].

To the best of our knowledge, there is no known vertical resistance (based on the R-gene) to inhibit or limit infection of *M. phaseolina*, but rather partial resistance which does not limit infection but reduces or compensates the damages.

5. CONCLUSION

Charcoal rot disease of cucurbits is caused by the pathogenic fungus *Macrophomina phaseolina*. It is a very important and serious disease on different vegetable crops, including all cucurbits species. The disease distributes in warm temperate and tropical regions of the world. Its symptoms are represented by appearing water-soaked lesions at the soil level and extend several centimeters up the stem. Lesions dried out progressively, turned tan, and cracked. Affected plants decline and die before harvest. Abundant black microsclerotia appear on the infected parts of the plants. Microsclerotia of the pathogen which found in soil, host root and stems are the main surviving propagules. Repeated freezing and thawing of soil, low carbon to nitrogen ratios in soil, and soil moisture content are the most important factors that significantly affect microsclerotia survival. Root exudates induce germination of microsclerotia and root infection of hosts. Charcoal rot disease can be managed and controlled by decreasing pathogen propagules in soil and host roots, avoiding favorable conditions for further pathogen survival and propagation by applying solarization and biosolarization, using grafted plants, and practicing many agricultural methods, chemical fungicides and biological control agents.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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