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MODULATING STRATEGIES: A TALE OF FUNGUS *MACROPHOMINA PHASEOLINA* (TASSI) GOID. INFECTION

Syeda Erum Razzaq*, Abdul Hakeem Shaikh and Muhammad Abid

Dr. A.G. Lab. of Aerobiology & Plant Pathology, Department of Botany, Federal Urdu University of Arts Science and Technology, Gulshan-e-Iqbal Campus, Karachi-75300, Pakistan.
*Corresponding Author: E-mail: syeda.er2@gmail.com

ABSTRACT

Food legumes such as grains and pulses are considered essential foods in most tropical and subtropical countries. Plant pathogens cause serious problems for the growth and survival of grain and pulse crops and there has been an estimated 44% loss in yield of these plants due to the diseases caused by plant pathogens. *Macrophomina phaseolina* is a soil borne fungus that causes charcoal rot disease. This fungal species persists in the form of small and black sclerotia dispersed in the soil because it does not survive in its mycelial state for more than 7 days. *M. phaseolina* can infect several hundred of plant species belonging to over 100 plant families. In Pakistan, *M. phaseolina* infection has been reported on more than 67 economically important plant species. The infection caused by *M. phaseolina* is difficult to control because of organism's thick wall hyphal mat and its sclerotia. However, there have been many attempts focusing on to reduce the fungal population by reducing the number of sclerotia in soil or to minimize the contact between host and inoculum. There are other approaches to modulate this soil-borne fungus population are fumigation, adding organic amendments, maintenance of high soil moisture content, solarization and the application of antagonists and fungicides.

Key words: *Macrophomina phaseolina*, food legumes, management strategies.

INTRODUCTION

Pulses and grains belonging to plant family Fabaceae are not only essential foods for human consumption but the fodder and grasses that are obtained from these crops are also attractive animal foods. In addition to their great value as food source pulse crops can also increase the fertility of soil because they produce nitrogen through nitrogen fixing rhizobacteria and thus minimizing the use of nitrogen fertilizer (Iqbal, 2010).

In Pakistan, major pulse crops are winter pulses (chickpea and lentil) and summer pulses (mung and mash bean). Although there are many biotic and abiotic factors that are responsible for the reduced yields of mung and mash bean in Pakistan, the plant pathogens are the biggest threats for the plants growth and survival; there have been an estimated 44% yield reduction in pulse crops because of pathogens infection (Bashir and Malik, 1988). Among the diseases, charcoal rot caused by *Macrophomina phaseolina* (Tassi) Goid, plays a major role in reducing crop yields especially in the arid areas of the world (Hoes, 1985).

Macrophomina phaseolina is a soil-borne fungus that causes root rot, stem rot and fruit rot diseases in around 500 species of plants (Sinclair and Backman, 1986; Khan, 2007) belonging to over 100 plant families (Mihail and Taylor, 1995). In Pakistan, *M. phaseolina* has been reported infecting more than 67 economically important plants species (Shahzad *et al.*, 1988). Cook *et al.* (1973) have found the charcoal rot infestation in certain individual fields of Nebraska to be high as 70% in corn and 80% in sorghum and the annual loss of sunflower caused by this fungus has been estimated to be 12% (Kolte, 1985). In addition, root rot of cotton caused by *M. phaseolina* is considered as a serious problem in cotton fields in Pakistan (Ghaffar *et al.*, 1969).

M. phaseolina survives in its mycelial state in the soil for about 7 days (Ghaffar, 1968; Meyer *et al.*, 1973). The fungus exists in the form of small and black sclerotia, which are formed on the tissues of various hosts and later spread in the soil through tillage operation (Cook *et al.*, 1973). *Macrophomina phaseolina* shows a great morphological variability, which increase its adaptability to survive in diverse environmental conditions (Kunwar *et al.*, 1986; Mihail and Taylor 1995; Srivastava and Singh 1990). Because *M. phaseolina* can survive in varied environment it requires varieties of different management techniques such as organic amendments, integrated control, biological control and crop rotation (Sheikh and Ghaffar, 1980) to control the disease caused by this organism. This review provides insight into the taxonomy, physiology, and biology of *Macrophomina phaseolina* add to our knowledge on the impact of the various diseases caused by this fungal pathogen on plant health in general and how to control or modulate its wide spread distribution in particular.

Macrophomina phaseolina taxonomy

According to recent phylogenetic data *M. phaseolina* is placed under Botryosphaeriaceae family (Crous *et al.*, 2006). However, over the past 100 years its taxonomic status has been revised at multiple times. In 1923, Petrak, establish the genus *Macrophomina.* There is a mycelial phase of this fungus called *Rhizoctonia bataticola* (Taub) Butler and there is a pycnidial phase called *Macrophoma phaseolina* (Tassi) and then Maublanc suggested a different name for species as *phaseoli).* Finally, in 1927 Ashby recognized it as the binomial species *Macrophomina phaseoli* (Maubl.). Later, in 1947 Goidanich changed the binomial *Macrophomina phaseoli* to *Macrophomina phaseolina* (Tassi) Goid. Currently, the correct taxonomic and officially recognized name this fungus is "*Macrophomina phaseolina* (Tassi) Goid." *Macrophomina* is a monotypic genus, composed of only one species, "*phaseolina*" (Sutton, 1980).

Macrophomina phaseolina diseases cycle and symptoms

Macrophomina phaseolina infect a wide varieties of plant species that includes fruit trees and weed species (Songa and Hillocks, 1996), softwood forest (McCain and Scharpf, 1989), legumes and vegetables like cotton, common bean, cowpea, soybean, sorghum, corn and peanut (Dhingra and Sinclair, 1977; Bouhot, 1967; 1968; Adam, 1986; Gray *et al.*, 1990; Hall, 1991; Diourte *et al.*, 1995) are also favorable hosts of this pathogen and its infection can result in seedling blight, root rot and stem rot on the infected plants. During adverse environmental conditions the sclerotia of *M. phaseolina* can survive in the tissues of host's root and stem (Cook *et al.*, 1973; Meyer *et al.*, 1973; Short *et al.*, 1980).

The primary inoculum and main surviving propagules, which infects host tissues and plant seeds are microsclerotia that are already present in the soil (Bouhot, 1968; Dhingra and Sinclair, 1977; Abawi and Pastor-Corrales, 1990). Initially, disturbance in the intercellular spaces of the epidermal cortex has been appeared due the penetration of fungal mycelium into the primary roots of host plant which may cause death of affected plantlets. Later, the microsclerotia has been formed in the vessels due to the intracellular growth of fungal hyphae in xylem tissues (Mayek-Pérez *et al*., 2002; Short *et al.*, 1978). After successful infection, necrotic areas can be seen on branches as well as on the stems of the infected plants. The severity of infection and the production of fungal toxins phaseolinone can cause premature death of plant (Bhattacharya *et al.*, 1994). The main propagation of pathogen is the mycelium and microsclerotia on the infected plants. Microsclerotia are released into the soil as a result of root decay and plant debris. The survival of ther propagules are affected by many factors including maintenance of soil moisture content, low C:N ratio, thawing and repeated freezing of soil (Dhingra and Sinclair, 1974; Dhingra and Sinclair, 1975).

Macrophomina phaseolina control measures

- **Cultural Practices:**

Cultural practices are those activities in agriculture that involves increasing the health of crop and livestock and decrease the production of weeds. These cultural practices may be categories into pre-planting operations, planting operations and post-planting operations. The combinations of cultural practices offer effective plant disease management. For instance, crop rotation and shuffling in crop planting time (Bristow and Wyllie 1975), usage of lime and fertilizer, organic amendments and deep ploughing (Collins *et al.*, 1991), sowing density and irrigation (Ploper *et al.*, 2001), soil application of zinc along with *Bradyrhizobium japonicum* and *Trichoderma viride* (Ansari, 2010) are being used to manage *M. phaseolina* infection on various cropping system.

According to Csondes *et al*. (2008) increase supply in nitrogen:phosphorus:potassium (NPK) play an important role to manage charcoal rot of soybean. In 2007, Ndiaye observed that crop rotation of *Vigna unguiculata* with non-host crop such as *Digitaria exilis* and *Pennisetum glaucum* for 2–3 years is considered necessary to lower *M. phaseolina* infection levels in severely infested fields. No-tillage system never support the growth of *M. phaseolina* and show less sclerotial population of *M. phaseolina* in root and stem tissues of soybean as compare to the conventional tillage system (Mengistu *et al*., 2009).

Solarization:

In 1980, Katan and his co-workers suggested that solarization was a feasible method to manage soil-borne pathogens. They observed the effect of solarization against soil-borne pathogens of onions in two different experimental fields at Yotvata (hot region) and at Gefen (cooler one). In both fields, there was 73-100% reduction in the severity of pink root disease during 6-7 months of growth. Furthermore, there is 109-125% increase in yields of the plant at Yotvata and 59-62% at Gefen as compare to control. However, Mihail and Alcorn (1984) conducted experimental trails during spring, summer and fall with 51-µm clear polyethylene tarp on naturally and artificially infested soil with *M. phaseolina* to observe the effect of soil solarization and found that solarization was not effective in suppressing *M. phaseolina* population. Later Ndiaye (2007) found that the combination of solarization and organic amendments not only reduced *M. phaseolina* infection but also increased yield of Cowpea (*Vigna unguiculata* (L.) Walp.).

Organic Amendments:

Soil amendments are added to improve the structure of the soil and also improves the fertility of soil. Organic soil amendments made by naturally occurring products. The most common examples of organic amendments are straw, leaves, biosolids, compost, manure and peat etc. Rathore (2000) used animal manure or farmyard manure, neem and mustard cake as organic amendment to control charcoal rot disease caused by *M. phaseolina* infection on mothbean. In a separate study, Lodha and collegues (Lodha *et al.*, 2003) found a significant 63-72% reduction in *M. phaseolina* population and increased crop when they used Pearl millet compost with soil amendment in cluster bean plants. In 2007, Ndiaye amended the field with 6 metric tons of compost per hectare and noted substantial reduction in charcoal rot disease and increased cowpea yield.

Soil Moisture Content:

Soil moisture contents are of great importance because it disturbed the heat sensitivity of *M. phaseolina*. Lodha *et al.* (2003) observed that within 90 days there was 13% reduction in the viable propagules in *M. phaseolina* infested dry soil by sub-lethal heating (45–55 °C), while one summer irrigation without sub-lethal heating caused 34% reduction in *M. phaseolina* propagules, when it was combined with 60 days of sub-lethal heating the reduction improved to 43.3%. They also examined the effect of *Brassica* amendments to the irrigated soil and observed the significant reduction (60.4–71.6%) in counts of *M. phaseolina* however this reduction improved to 89.4–96.1% when combined with sub-lethal heating. One summer irrigation was enough for the reduction of *M. phaseolina* by 25–42 % (Lodha and Solanki, 1992; Lodha, 1995).

Fumigation:

Soil fumigation is the chemical treatment of soil to control pest by using pesticides, these pesticides converted into a volatile gas which has ability to spread into the soil through pore space and play an effective role against soil-borne pest to control diseases. Soil fumigation, such as methyl bromidation fumigation is highly effective in the control of viable microsclerotia population and has been found to prevent the incidence of *M. phaseolina* infection and increase soybean yield (Watanabe *et al.*, 1970). Moreover, the fumigation of soil with sodium methyl dithiocarbamate has been found to reduce the pathogen populations on soybean (Kittle and Gray, 1982).

Use of Antagonists:

In phytopathology antagonist is an organism that have ability to suppress or inhibit the growth and activity of another organism or plant pathogen. In *in-vitro* test of soybean several antagonistic, rhizosphere-inhibiting fungi and bacterial endophytes were identified (Senthilkumar *et al.*, 2009). Farm practices through tillage practices may directly induce *Trichoderma* spp. populations and indirectly reduce the soil-borne pathogens including *M. phaseolina* (Baird *et al.*, 2003). In 2010, Dawar and her associates examined the antagonistic effects of *Bacillus subtilis* on *M. phaseolina.* They observed that *Bacillus subtilis* showed zone of inhibition of *M. phaseolina* by dual culture plate method while seed dressing and soil drenching with *B. subtilis* were effective methods to control the infection by *M. phaseolina* on cow pea and mash bean plants.

In 2004, Hashem used three antagonists (*Epicoccum nigrum, Paecilomyces lilacinus* and *Trichoderma harzianum*) against *M. phaseolina* on soybean and noted that *Epicoccum nigrum* and *Paecilomyces lilacinus* produced an inhibition zone, while *T. harzianum* showed overgrowth. In other study, Ehtesham-ul-Haque and colleagues (2007) found that fungal infection caused by *M. phaseolina* infection on soybean was reduced by 14–100% by seed treatment with *Pseudomonas aeruginosa*. Recently, Akhtar and colleagues (2011) found that treatment of seeds with two fungal species *Arbuscular mycorrhizal* fungi and *Rhizobium* spp. enhanced the plant growth and controlled fungal infection on host plants such as chickpea, pigeon pea and lentil etc. In Pakistan *Rhizobium* strains also reported to have suppressed the cultural growth of *M. phaseolina* (Zaki and Ghaffar, 1987).

To achieve sustainable agriculture, application of AM fungus with *Rhizobium* sp. has been successful because AM fungi induces the absorption of water and soil nutrients, whereas *Rhizobium* sp. produces antibiotics and phytoalexins in addition to its role in nitrogen fixation (Akhtar *et al.*, 2011).

Use of Fungicides:

Fungicides have been used routinely to control fungal diseases. Also, many studies have been conducted to investigate the effect of fungicides against the different isolates of *M. phaseolina* (Al-Beldawi *et al.*, 1973). Although control of *M. phaseolina* population by chemical fungicide is effective it is not preferred method because of environmental concern (Pearson *et al.*, 1984). Prajapati *et al.* (2003) applied carbendazim in combination with Thiram on chickpea and observed highest seed germination percentage and lowest root rot incidence and Rathore and Rathore used Bavistin 50 WP [carbendazim] on moth bean seeds and found significant suppression of *M. phaseolina* infection on this plant (Rathore and Rathore, 1999). Treatment of seeds by fungicides also help to reduce the losses caused by *M. phaseolina* in many crops. Thiophanate-methyl and furcarbanil have highest degree of control against *M. phaseolina* on soybean (Vir *et al.*, 1972).

Use of Plant Extract:

There are some plants, which have high antimicrobial ⁄ antifungal activities (Bisht *et al.*, 2010). For instance, soaking plant seeds in ginger, garlic and neem extracts for 5 min the infection of *M. phaseolina* was completely inhibited on soybean (Hossain *et al.*, 1999). In 2003, Arora and Kaushik found that dry extracts of *Cleome viscoasa* and *Mentha longifolia* in hot water and the methanol extracts of *Berberis aristata*, *Conyza bonariensis, Cleome viscoasa, Lantana camera* and *Vitax negunda* completely reduced *M. phaseolina* population (Arora and Kaushik, 2003). Use of plant extract is an lternate source of chemical fungicides, as their highly negative impacts while plant extracts are eco-friendly and showed antagonistic activity against variety of diseases.

Conclusion

M. phaseolina is a polyphagous and unspecialized pathogen and many studies conducted related to its host specification, but studies did not reveal clear-cut evidence about host specialization of *M. phaseolina* and can causes serious injuries to the number of crops including food legumes and these injuries may cause the death of plant. As a result of plants death, the yield of crop become highly affected. *M. phaseolina* is very resistant fungus and hard to manage due to its ability to survive in adverse environmental conditions. The disease is more severe when plants are under stress from moisture or nutrients, excessive plant densities, soil compaction, improperly applied pesticides, nematodes or other pathogens. Many other reports indicated to manage the diseases cause by *M. phaseolina* like the disease could be reduced by the applications of organic manures and micronutrients coupled with irrigations, integrating rotations, soil fertility and sowing density.

In recent era, genetic engineering techniques get more attention in order to cure the disease. One of the modern developmental diagnostic tools in these techniques is automatic purification of specific proteins and nucleic acids from pathogen because the accurate identification of pathogen on the basis of biology is necessary to manage diseases. This information can be used to improve disease control decision making. In future, the development in applications of molecular diagnostic methods will increase the adoption of new technologies for the diagnosis and study of plant disease that will be a great sign for the betterment of disease management in plants.

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