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Review Article

Macrophomina phaseolina as causal agent for charcoal rot of sunflower

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Abstract

Macrophomina phaseolina has a wide host range and is responsible for causing losses on more than 500 cultivated and wild plant species. So far in Pakistan it has been reported to cause disease on 67 economic hosts including field crops, pulses, flowers and vegetable have been reported. Infection on sunflower was first reported from Sri Lanka in 1927 and in 1982 it was reported from sunflower field in Pakistan. The fungus is reported to be soil, seed and stubble borne. The fungus can survive for more than 10 months under dry soil conditions. The severity of the disease is directly related to the population of viable sclerotia in the soil. The pathogen generally affects the fibrovascular system of the roots and basal internodes. The parasitic fitness of a facultative soilborne pathogen before invading the host is linked with its ability to compete for its survival, utilization of organic sources and colonization in the host root rhizosphere by competing other microorganism in the vicinity.

Keywords: Charcoal rot, *Macrophomina phaseolina*, sunflower.

Taxonomic Description of *Macrophomin phaseolina* (Wheeler, 1975)

Division	*Eumycota*
Sub Division	*Deuteromycotina*
Class	*Coelomycetes*
Order	*Sphaeropsidales*
Family	*Sphaeropsidaceae*
Genus	*Macrophomina*
Species	*phaseolina*

Macrophomina phaseolina infection on sunflower was first reported from Sri Lanka in 1927, It was then reported from Uruguay, Australia and Yugoslavia in1966 Argentina and Senegal (1967), Hungary (1970), USA(1971),India (1973), France, 1976, Egypt (1980) and Pakistan, 1982 (Bhutta, 1997).

The fungus is reported to be soil, seed and stubble borne. The evidence suggests that it is primarily a root inhibiting fungus and produces tuber or cushion shaped 1-8 mm diameter black sclerotia. These sclerotia serve as a primary means of survival (Smith, 1969; Mirza, 1984; Kaisar, *et al*., 1988). The sclerotia float freely on soil surface when field is flooded for irrigation and become primary inoculum for emerging seedlings. Additional sclerotia become dislodged and rise to the water surface in the flooded seed bed or any other factor, such as water wave action due to wind that disturbs the surface layer during growing season (Keim and Webster, 1974; Webster *et al.*, 1976). The fungus can survive for more than 10 months under dry soil conditions. The severity of the disease is directly related to the population of viable sclerotia in the soil. However, mycelium of *M. phaseolina* in the soil is not considered as a major source of inoculum (Norton, 1953; Smith, 1969; Meyer *et al.*, 1974). In Pakistan soils up to 25 sclerotia of *M. phaseolina* in 1g soil have been reported and evidence indicate that a single sclerotium of the fungus can cause death of the plant in its susceptible host. Pre-emergence mortality is directly related with the ability of the strain of *M. phaseolina* to infect the embryo (Burney *et al*., 1984, Dhingra and Sinclair, 1978, Francl *et al*., 1988). Strains of the fungus responsible for pre- emergence mortality can penetrate 5-10 µ intact thick membrane. The strains of the fungus that fail to cause pre-emergence mortality move towards upper parts of the plant. Pathogenicity of such strains is linked with the degree of nutritional compatibility with the host tissue (Khan *et al*., 2000).

M. phaseolina has a wide host range and is responsible for causing losses on more than 500 cultivated and wild plant species (Indera *et al.*, 1986). So far in Pakistan, 67 economic hosts of *M. phaseolina* including cotton, rice, maize, cucurbits, okra, and wheat have been reported (Mirza and Qureshi, 1978; Shehzad *et al*., 1988). Wide host range of *M. phaseolina* suggests it as non-host specific fungus. Physiological specialization of the fungus is not well demonstrated. High level of variation in morphology, physiology and pathogenesis has been reported even when isolated from different parts of the same plant (Dhingra and Sinclair, 1973).

The pathogen *M. phaseolina* generally affects the fibrovascular system of the roots and basal internodes, impends the transport of nutrients and water to the upper parts of the plant. Progressive wilting, premature dying, loss of vigor, and reduced yield are characteristic features of *M. phaseolina* infection. The pathogen is also responsible for seedling blight, damping off, root rot, basal stem rot and early maturing of sunflower crop. But the characteristic symptoms that appear after flowering are grey black discoloration and shredding of plant tissue at the stem and top of the taproot with getting hollowing of the stem. When the epidermis is removed, minute black microsclerotia may be so numerous as to give a greyish black look to the tissue (Sinclair, 1982; Yang and Owen, 1982; Hoes, 1985; Kolte, 1985).

Pathological constraints of sunflower

Sunflower (*Helianthus annuus* L.) belongs to the family *Asteraceae* and genus *Helianthus*. The common sunflower comprises of three main races: namely, *H. annuus* ssp. *lenticularis* (wild), *H. annuus* ssp *annuus* (weed) and *H. annuus* ssp *macrocarpus* cultivated. *H. annuus* ssp *macrocarpus*, the giant sunflower, is cultivated for edible seed. Edible sunflower is susceptible to diseases, whereas its wild parents have resistant genes against diseases. Sunflower is native to the North America and being successfully cultivated under diversified geographical and agro-ecological zones.

Diseases are serious threat for the sunflower crop throughout world. It has been estimated that diseases can cause an average annual loss of 12% in yield from nearly 12 million hectares of the world (Zimmer and Hoes 1978; Kolte 1985). The incidence and severeity of diseases are linked with the climatic factors and cultural practices. Among diseases, rust, verticilium leaf wilt, downy mildew, sclerotium wilt and charcoal rot are of worldwide occurrence. Prevalence or distribution of the disease is linked with the climatic factors, cropping pattern, and cultural practices. In general diseases cause 10-15% net loss but under favorable conditions for the outbreak and development of the pathogen, they may claim failure of the crop (Sackston 1981; Xiaojian *et al.*, 1988).

Besides general prevalence of the diseases, some countries are also facing specific pathological threats to the sunflower crop. Madjidiech (1988) reported 20% yield losses due to *Phomopsis helianthi* and *Puccinia helianthi*. In Nigeria and Egypt, fungal population was more than other organisms on above and under ground parts of sunflower. The major fungal pathogens were *Alternaria*, *Cercospora, Chlamydosporium, Curvularia, Fusarium sp.* and *Macrophomina phaseolina* (Satour, 1984; Ado and Tanimu, 1986; Ado *et al.*, 1988).

Bhutta *et al.*, (1997) conducted a systematic survey during 1991-1992 on frequency and distribution of various diseases on sunflower in Pakistan. He reported more than 16 diseases including charcoal rot (*M. phaseolina*), head rot (*Sclerotina sclerotium* and *Rhizopus* sp.), stalk rot (*S. sclerotium*), collar rot (*Sclerotium rolfsii*), black stem rot (*Phoma oleceara*), bacterial rot (*Erwinia caratovora*), leaf spots (*Alternaria halianthi*, *A. tenius* and *Septoria helianthi*), rust (*Punccinia helianthi*) and powdery mildew with high level of occurrence in the country. However, in Pakistan comprehensive information on distribution and severeity of sunflower diseases in various agro-ecological zones is not available.

Until now more than 25 diseases have been reported on sunflower in China (Xiang *et al*., 1988). *Alternaria helianthi, Orobanche coerulescens, Sclerotinia sclerotium and Septoria helianthi* cause 10-15% yield losses. Madjidiech, (1988) reported 20-50% losses due to *Plasmopara helianthi* on sunflower in Iran. Mirza and Beg, (1983) conducted first survey of the sunflower crop in the central and northern areas of Pakistan during 1982. In this survey, *Macrophomina phaseolina* (charcoal rot) and *Rhizopus spp.* (head rot) were reported as most destructive on sunflower crop. Mirza, (1984) reported yield losses due to *M. phaseolina* up to 90% in Pakistan on sunflower in 1984.

Masirvic *et al*. (1987) reported eleven diseases of sunflower from Punjab, Sindh and NWFP provinces. Among these diseases, charcoal rot (*M. phaseolina*), head rot (*Rhizopus* spp.) and sclerotina rot (*Sclorotina sp.*) were of major occurrence. Bhutta *et. al.*, (1995), conducted comprehensive survey on distribution of pathological problems of sunflower at farmer field in various agro-ecological zones in Pakistan during 1991-92. So far 16 diseases on sunflower including 12 fungal, 2 bacterial, 1 viral and one nematode have been reported in Pakistan, among them charcoal rot, head rot, bacterial blight and *Alternaria* leaf spot were reported as serious threats to sunflower (Ahmed *et al.*, 1991; Bhutta *et al*., 1995).

Charcoal rot of sunflower was reported for the first time from Faislalabad (Mirza, 1984) and later from other areas of Punjab, Sindh and NWFP provinces as a serious threat to sunflower (Mirza and Beg, 1983; Steven *et al.*, 1987). In Pakistan charcoal rot is associated at maturity of plants under water stress. Favorable conditions for the

development of the pathogen may lead to the death of plant or failure of the crop. Up to 60% yield losses due to charcoal rot have been reported (Steven, *et al*., 1987). High level of variation in fungus, soil borne habitat, and good survival ability of the sclerotia makes its chemical control difficult and uneconomical. Therefore, the most appropriate approach to combat the pathogen is the use of resistant varieties. So far no commercial sunflower cultivar has been reported for resistance against *M. phaseolina* (Gul *et al*., 1989; Ahmed and Burney, 1990; Hafeez and Ahmad, 1997).

Global distribution and economic importance

Macrophomina phaseolina causal agent of charcoal rot is a serious threat for sunflower crop especially in the arid regions of the world (Hoes, 1985). Yield losses claimed by charcoal rot in Spain, United States, Uruguay and Soviet Union up to 25% has been recorded however under favorable conditions for the growth and development of the *M. phaseolina* total failure of the crop in specific areas have been recorded (Sackston, 1959; Orellana, 1971; Tikhonov *et al*., 1976; Jimens *et al*., 1983)

Charcoal rot is of great economic importance in arid areas of the world. It causes decrease in stem height 3.12, girth 36.44, root 72.56 and head weight 10.77% (Raut, 1983; Kolte, 1985). Severeity of the disease is characterized by drought and high temperature. However, high losses have been reported on availability of low relative humidity and high atmospheric temperature at flowering stage of the crop (Dhingra and Sinclair, 1973; Tikhonov *et al.*, 1976).

Distribution of charcoal rot in Pakistan

Mirza and Beg (1983) reported more than 10 diseases on sunflower in Punjab and stated *Macrophomina phaseolina* as the most serious problem in the areas of drought stress and high temperature. The other major problems on sunflower crop were Stalk rot (*S. sclerotium*), head rot (*Rhizopus spp. and S. sclerotium*) leaf spot (*A. helianthi and S. helianthi*) are major pathological constraints and stand next to charcoal rot in their pathological status. Whereas powdery mildew (*Erysiphe cichoracearum*), and rust (*Puccinia helianthi)* were of lesser occurrence. Charcoal rot is an important threat to sunflower crop in Pakistan Under favorable conditions 90% prevalence of charcoal rot with severe intensity has been reported from major sunflower growing areas of Sindh and Punjab (Rana, 1999).

Epidemics of charcoal rot

Severity of infection caused by a soilborne pathogen depends upon its parasitic fitness in soil ecology. The parasitic fitness of a facultative soilborne pathogen before invading the host is linked with its ability to compete for its survival, utilization of organic sources and colonization in the host root rhizosphere by competing other microorganism in the vicinity. Nature of soil is also an important factor in controlling the activities of a fungus due to its adsorption capacity in utilizing the soluble nutrients. The activity of exudations from the sclerotia and utilization of soil nutrients also explain the pathogenic importance of a soilborne fungus (Filnow and Lockwood, 1983; Mazzola *et al.*, 1996).

Climatic conditions like temperature, atmospheric humidity, and available moisture play a significant role in activation and multiplication of *Macrophomina phaseolina*. Its epidemiological requirements may vary greatly for invading the host and development of visible symptoms. At seedling infection stage *M. phaseolina* invades the host very rapidly and establishes itself in the host within 24 to 48 hours, when high moisture and low temperature prevails. If the invaded plant survives from the seedling mortality then the fungus moves to the above ground parts at a very slow rate. The typical symptoms of the disease become visible at grain formation stage when low moisture and high temperature exist, however, the range of relative humidity and temperature requirement may vary, depending upon the nature of isolate, climatic region and host cultivar (Ahmed, 1996; Edmunds, 1964).

Variation

Variation is simply a product of spontaneous mutations, molded by recombination and natural selection. It is a constant phenomenon and may occur in any population. The rate of occurrence of mutation depends upon species, age, and environment. The variation in *Macrophomina phaseolina* may have a relation, with geographical origin and source (Meyer *et al.*, 1974; Harlton *et al.*, 1995). The qualitative differences in *Macrophomina phaseolina* are related to the host specificity of the fungus. The host specificity of the fungal isolates varies from crop to crop: i.e., it may show host-specific behavior on one crop and may not on the other. The in vitro cultural and pathogenic differences are highly variable and are very difficult to quantify for adequate classification (Farrara *et al.*, 1987; Clude and Rupe, 1991).

Physiological specialization in *Macrophomina phaseolina* is not well demonstrated and the fungus is known to have high degree of variation in its morphological, cultural and pathological properties even when isolated from different parts of the same plant (Dhingra and Sinclair, 1978). The genetic variation within a fungal population has been correlated with many factors, pathogen exhibits variation due to uniformity of its substrate and environmental experience. The degree of sexual reproduction within a population or specie is also related to genetic variation (Karik, 1986; Newton, 1987; Kendrick, 1992). The unstable B chromosome may be one of the mechanisms for generating variation in fungi (Miao *et al.*, 1992)

Due to the high degree of genetic variation in the pathogen, the most appropriate approach to combat the disease is the use of resistant variety (Ahmed and Burney, 1990). However, no resistant commercial sunflower germplasm is available in Pakistan (Gull *et al*., 1989; Ahmed and Burney, 1990; Hafeez and Ahmad, 1997). The high degree of variation in the pathogen makes the efforts for evolving resistant hybrids of sunflower very difficult, because breeders have to develop resistance against a large number of pathogen population (Venuto *et al.*, 1995). Variability in resistance may be attributed to the variation in the host or pathogen. The large-scale variation is observed in pathogen population (Horst, 1965). Therefore, knowledge about the variation in host and pathogen are prerequisite for successful breeding program (Prota-Puglia *et al.*, 1996).

Variation in mechanism of infection

The plant cell wall is a complex structure of polymers which surrounds the cell. *Macrophomina phaseolina* enters the host tissue either by exerting mechanical pressure by their growing germ tubes or dissolve the cell wall through the secretion of toxins or enzymes. Fungus can penetrate intact wax membrane up to 5-10 µ. Degradation of cell wall by *M. phaseolina* is due to the enzymes polyglacturonase and cellulase. Development of intercellular mycelium and its close association with cell wall in the cortex are compatible with intensity of polyglacturonase. The early incidence of infection is favored by soil moisture contents that did not correspond to the drought condition and the high temperature consistently inhibits the route of infection (Wheeler, 1975).

Variation in the process of disease development

For successful development of charcoal rot disease of sunflower, virulent isolate of *Macrophomina phaseolina* accompanied by low moisture and high temperature are essential. The severeity of infection depends upon relative humidity, temperature, nature of isolate, climatic region, and host cultivar (Edmunds, 1964; Ahmad, 1996).

Under field conditions *M. phaseolina* establishes itself during early stages of plant growth but symptoms of the disease don't appear until maturity, when plant contains lower level of moisture percentage. Appearance of typical symptoms at crop maturity suggests the possibility of latent quiescent infection because plant showing good growth and high vigor during early stages of its growth shows severe disease symptoms at maturity (Meyer *et al.*, 1974). The infected plants show early maturity, reduced head size, and fewer number of grains setting. Visibility of the symptoms depends upon the severity of infection. The fungus primarily invades secondary and tertiary roots then travels to primary root. The fungus infects fibro-vascular system of root and basal internodes impending the transport of nutrients and water supply to the above ground parts of the plant. Due to destruction of root system and blockage of food supply channel infected plant loses its tenderness therefore can easily be pulled out, but without secondary or tertiary roots. In cases of severe infection charcoal rot affected field looks burned (Ahmad and Burney, 1990; NODP, 1994).

Variation in symptoms of charcoal rot under field conditions

Charcoal rot, which was found as one of the major pathological constraints to sunflower, exhibited with wide range of variation in disease severity. Depending upon the severity of disease a wide range of variation in symptoms of charcoal rot on individual sunflower plant or crop stand were observed. It may be due to the soil type, inoculum density, climatic conditions of the area and genetics in host germplasm as well as pathogen itself. The other contributing factors likely were pathogen inoculum level in the field and climate of the region. In coastal areas of Sindh province, the plant stem was brown without any type of black spots or streaks on bark of stem and it was very difficult to pull the plant out of the soil. When the plant was successfully pulled out, there was no destruction of root system. On dissecting the main root, scattered black sclerotia of *M. phaseolina* were seen. In the cotton growing areas of southern Punjab, which are not in the seepage zone of canal, river or dam, visible symptoms were not greatly different from coastal Sindh. But severity of the disease was somewhat higher than coastal areas of Sindh province. However, the

collar region of the stem was black and rest of the stem had a blackish brown look. A partial destruction of secondary and tertiary roots was common. Sclerotia count of *M. phaseolina* in the primary root was higher than coastal Sindh. Symptoms of the disease observed in rice zone of Punjab and NWFP province were similar and were more prominent and severe than rest of the country. Because climatic conditions of the root zone was favorable for development of fungus in the host tissue one-third to one-half of the stem was turned blackish brown and plant was easily pulled out. Destruction of adventitious roots was more prominent and sclerotia count in roots was higher (Khan, 2002).

Symptoms of the disease observed on sunflower in the seepage zone of canal, river and dam in Punjab and NWFP province were more severe than those observed in the rest of the country. In these areas, the pith of the plant was filled with black sclerotial powder. Due to lost turgidity the plant was not able to bear the floral head even though it was smaller in size and light in weight. Root systems of the plants were totally destroyed and fields had a burned look. Due to the heavy amount of sclerotia, certain patches of the light colored soil had a blackish look.

The survey conducted on the investigations of problems highlighted the need to conduct detailed studies towards the solution of charcoal rot disease. Charcoal rot is a serious threat for sunflower crop around the globe, especially in the temperate regions. In Pakistan it was reported on sunflower crop in 1996 (Khan *et al.*, 1999). Our investigations revealed that charcoal was identified as a most serious threat for the crop throughout the country. The disease is caused by *Macrophomina phaseolina*, which is primarily a soilborne fungus. Due to high degree of genetic variation in the pathogen, cultivation of resistant varieties is the most economical and practical approach. Other remedies of the disease are either uneconomical or cannot be applied under farmer field conditions. However no resistant commercial sunflower germplasm is available in Pakistan (Gull *et al.*, 1989, Hafeez and Ahmad, 1997). High degree of variation in *M. phaseolina* has been reported in the fungus even when isolated from different parts of the same plant. The high degree of variation in the pathogen makes the efforts for breeding resistant hybrids of sunflower very difficult, because breeders have to develop resistance against a large number of pathogen populations (Venuto *et al.*, 1995). The knowledge about the variation in host and pathogen is a perquisite for successful breading program (Porta-Puglia *et al.*, 1996). A series of experiments was designed to make detailed investigations on variation in *M. phaseolina* in relation to resistance against charcoal rot of sunflower. In this project entitled "Studies on variation in *Macrophomina phaseolina* in relation to resistance against charcoal rot of sunflower" all the parameters related to host, pathogen, and host-pathogen interaction under variable set of environments were quantitatively evaluated.

Development of pathogen in host system

Development of *Macrophomina phaseolina* in the vascular system of the plant is not in a systematic manner. Early incidence of infection is favored by soil moisture contents. The drought conditions and high temperature that did not prevail during root infection (Burton *et al.*, 1987; Raut, 1983; Raut, 1985). Bhutta *et al.*, (1995) studied the transmission process of *M. phaseolina* from root to upward growth of the sunflower and development of fungus. *M. phaseolina* established in the seedlings within 48 hours of entering in the host tissue. At cotyledon stage, seedling gets infected 3-7 days after sowing. Variation of host in disease severity is due to genetic variation, geographical origin or source of the isolate. First true leaves from 20-30% of plant yielded the pathogen after 7 days of growth but without any visible necrotic spots. The infection of stems at 1st node was evident after 60 days of germination. In case of severe infection profound sclerotia were found on bark of stem. The fungus was never isolated from the leaves above 3rd node, stems above fifth internode and flowers (Hodges, 1962; Dhingra and Sinclair, 1978). Underground symptoms start with development of lesions on the taproot and basal portion of the stems causing susceptible plants to wilt. Initial infection on above ground parts occurs at the base of the stem progressed with rapid drying of the leaves. Plant wilted at any stage from flowering to near maturity has significantly reduced yield (Prioletta and Bazzalo, 1998).

Perpetuation and over wintering

Positive correlation between inoculum level of *Macrophomina phaseolina* in the seedbed and disease severity has been reported. The fungus overwinter from one season to the next either as free sclerotia in the soil or in plant residue. Mechanical injury, high plant density, and insect attacks are considered as predisposing factors for the disease transmission (Ilyas *et al.*, 1975; Shiekh and Ghaffar, 1984; Dodd, 1980; Ahmed *et al.*, 1991; Cook *et al.*, 1973).

Host-pathogen interaction

Host - pathogen interaction determines the ability of host to bind a parasite and ability of parasite to injure the host. Whereas resistance and susceptibility are heritable qualities (Yang *et al.*, 1999). Certain new aspects of pathogenic relations of *Macrophomina phaseolina* and sunflower have been identified. Therefore, understanding the genetics, behavior of host and pathogen in the process of disease development and host -pathogen relationship are crucial for reliable breeding program for disease resistance. So far many attempts have been made in order to understand various aspects of host pathogen interaction involved in infection pathway. Host-pathogen parasitic compatibility in plant pathogenic fungi is related to their antigenic similarity. However, no common antigen is found between *Macrophomina phaseolina* and resistant cultivar of soybean (Farrara *et al.*, 1987 ; Limpert *et al.*, 1990; Jones *et al.*, 1996; Kema *et al.*, 1996).Extensive genetic variation and site-specific nature of *M. phaseolina* have made studies on genetics of charcoal rot resistance difficult. Therefore, genetics of resistance against *M. phaseolina* have not been clearly demonstrated and controversies are found in the findings of various workers. Resistance in sunflower genotype is a dominant character (Olaya *et al.*, 1996; Michel, 2000).It has been reported that resistant genes against *Macrophomina phaseolina* do not exist or are unknown. Whereas it has been reported that two dominant genes MP 1 control that impart resistance against *Macrophomina phaseolina* in peas and MP 2 and presence of these two genes in resistant cultivar is essential.

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