*Mycopath* (2003) **1**(1): 31-34

# Physiological studies on *Macrophomina phaseolina* (Tassi) Goid

**Shahid A. Khanzada, S.M. Iqbal and A.M. Haqqani**

*Pulses Programme, National Agricultural Research Centre, Islamabad*

## Abstract

*In vitro* physiological studies on mycelial growth of *Macrophomina phaseolina* (Tassi) Goid revealed that the fungus grew best on cornmeal agar out of five culture media that were tried. All the carbon sources were found to be more or less equally good while peptone was the best among the nitrogen sources. Growth of *M. phaseolina* was maximum at $30^0$C after 7 days of inoculation, which was reduced significantly below $20^0$C and above $35^0$C. All the tested pH levels (6.0 to 8.0) were found equally suitable for growth of fungus.

**Key words:** *Macrophomina phaseolina*, culture media, pH, carbon, nitrogen, mycelial growth.

## Introduction

Mashbean (*Vigna mungo* L.) is an important summer pulse crop in Pakistan. The yield of this crop in Pakistan is very low (504 kg/ha) due to several biotic and abiotic constraints (Anonymous, 2001). Among the biotic constraints, charcoal rot caused by *Macrophomina phaseolina* (Tassi) Goid (syn. *Sclerotium bataticola* Taub.), is of prime importance in reducing crop yield (Dhingra *et al*., 1978). This fungal pathogen causes seedling blight; root, stem and pod rot on more than 500 plant species (Sinclair, 1982) whereas at least 67 hosts have been recorded from Pakistan (Mirza & Qureshi, 1982; Shehzad *et al*., 1988). Charcoal rot caused by *M. phaseolina* is one of the most important diseases of field crops in arid regions of the world (Hoes, 1985).

Orellana (1970) reported that charcoal has inflicted significant losses to sunflower and Tikhonov *et al*. (1976) reported 18 to 64% yield reduction in sunflower by charcoal rot, but no such losses by this disease are documented in mashbean. Similarly, varietal screening against this disease in sunflower (Mirza *et al*., 1982; Hafeez & Ahmad, 2001) and sesame (Mirza *et al*., 1986) in Pakistan was conducted but such sort of study was lacking in case of mashbean.

Knowledge about the physiological factors affecting the pathogen and disease development is the prerequisite for designing the disease control strategies. Therefore, preliminary studies on some physiological aspects of this fungus were initiated.

## Material and Methods

Studies of the following physiological aspects of *M. phaseolina* were conducted *in vitro*.

**1. Effect of culture media**

Five culture media viz; Chickpea seed meal dextrose agar (chickpea seed meal 20 g, dextrose 20 g and agar 20 g), Potato dextrose agar (potato starch 20 g, dextrose 20 g and agar 20 g), cornmeal agar (cornmeal 20 g, dextrose 20 g and agar 20 g), Czapek Dox agar (sodium nitrate 2 g, potassium nitrate 1g, magnesium sulphate 0.5 g, potassium chloride 0.5 g, ferrous sulphate 3 g, sucrose 30 g and agar 20 g) and Sabouroud's agar (dextrose 40 g, peptone 10 g and agar 20 g) were used to find out the most suitable one for the mycelial growth of the fungus. Each culture medium was prepared in one liter of water and autoclaved at $121^0$C and 15 psi for 20 minutes. These were cooled to 45 $^0$C and then poured in 9 cm petri dishes for solidification.

**2. Effect of different Carbon and Nitrogen Sources**

Cornmeal medium (in one liter of water) was used as the medium for studying the effect of carbon and nitrogen sources.

**a) Nitrogen Sources**

Three nitrogen compounds viz; Potassium nitrates 10 g, Sodium nitrate 8.5 g and Peptone 2.5 g were amended in cornmeal agar medium.

**b) Carbon Sources**

Three carbon compounds viz; glucose 13.5 g, sucrose 12.5 g and starch 12.5 g were tried individually as a constitute of carbon source in cornmeal agar medium.

**3. Effect of different pH levels**

The test fungus was inoculated on cornmeal agar medium whose pH was adjusted to 6.0, 6.5, 7.0, 7.5 and 8.0.

**4. Effect of Temperature:**

The fungus *M. phaseolina* was inoculated in Cornmeal medium using five petri dishes for each temperature viz; 5, 10, 15, 20, 25, 30 and 35 $^0$C.

All these experiments were conducted in five replicates. Plates were inoculated by placing an equal amount of inoculum in the centre of the petri dishes. Plates were incubated at $30^0$C (except for the study of temperatures) when observations on radial growth were recorded after 7 days of inoculation.

## Results and Discussion

**1. Effect of culture media**

The results of the experiment revealed that the cornmeal medium was the best for the radial growth of *M. phaseolina* as this fungus gave maximum growth of 6.8 cm after 7 days of inoculation followed by Sabouroud's agar and Czapek Dox agar which showed growth of 5.5 cm and 4.0 cm, respectively (Fig.1).

**2. Effect of different carbon and nitrogen sources**

The results of this experiment indicated that all the carbon sources were equally good for growth of the fungus (Fig.2). The fungus may utilize certain simple form of complex carbon compounds and may convert complex carbon compounds into simple form which may be readily metabolized (Bais *et al*., 1970).

As is evident from Fig.3, peptone was found to be the best source of nitrogen for *M. phaseolina*. It was followed by Potassium nitrate ($KNO_3$). On potassium nitrate, the growth of fungus was 8.2 cm after 5 days of inoculation.

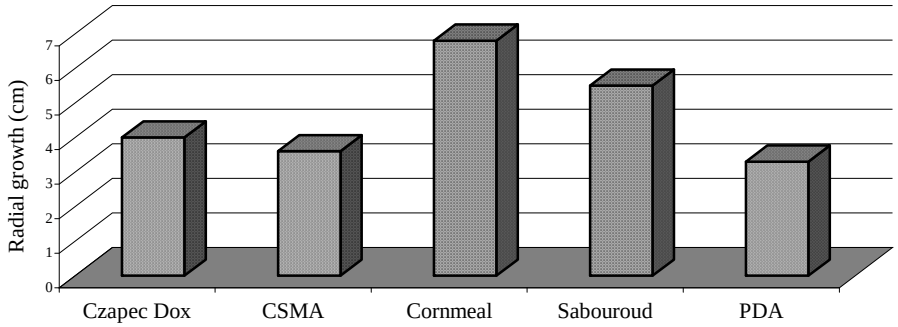


**Fig-1: Effect of different culture media on the mycelial growth of *M. phaseolina*.**

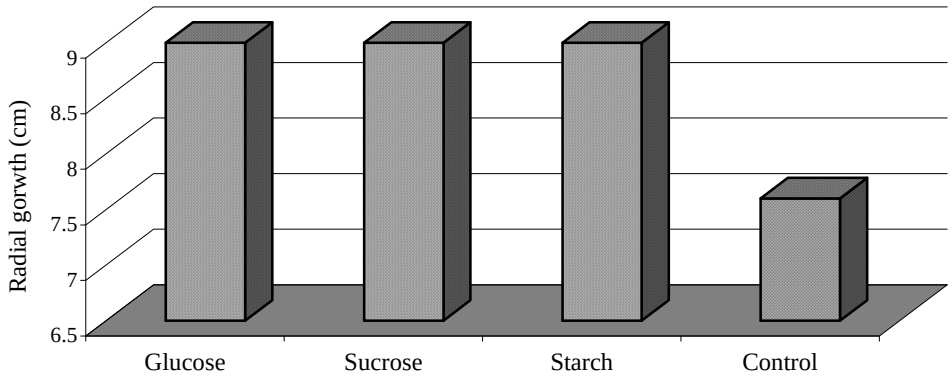


**Fig-2: Effect of Carbon sources on the mycelial growth of *M. phaseolina.***

**3. Effect of different pH levels**

All the pH levels tested were found equally good for the mycelial growth of the fungus (Fig.4) as the maximum average growth of 9.0 cm was recorded after 7 days of inoculation.

**4. Effect of Temperature**

As evident from Fig.5, the fungus grew at all the temperatures tried for the radial growth except $5^0$C. However, the growth of fungus was drastically reduced below $20^0$C and started to decline above $30^0$C, as these temperatures did not favour much growth of the fungus. It was observed that at $30^0$C, the fungus had maximum diameter (9.0 cm) while at $25^0$ C, it was 8.7 cm and at 35 $^0$C it was 7.9 cm after 7 days of inoculation. On the contrary, minimum growth of fungus was 2.4 cm at $10^0$C. Growth of the fungus was completely retarded at $5^0$C. Chandra and Purkayastha (1977) also reported that the suitable temperature for the growth of this fungus is 25-$30^0$C.

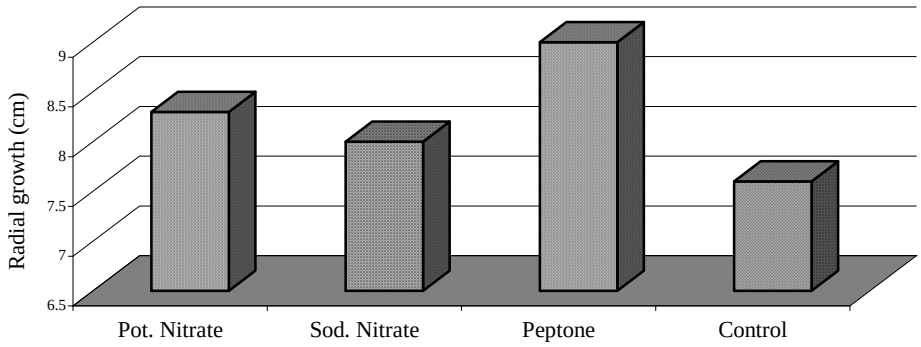


**Fig-3: Effect of Nitrogen sources on the mycelial growth of *M. phaseolina*.**

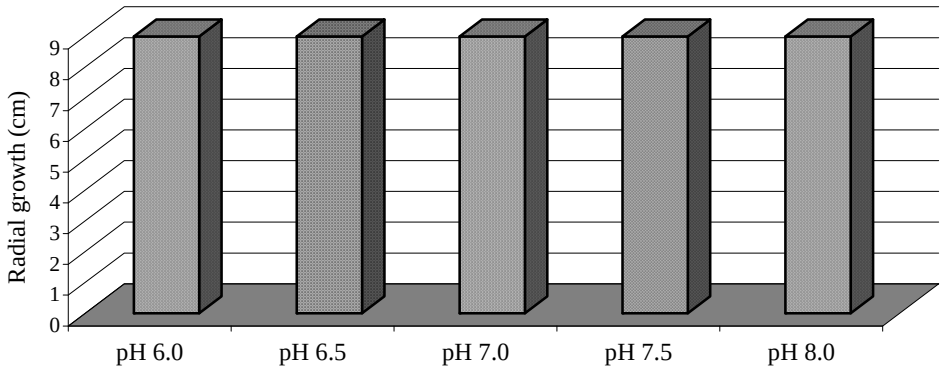


**Fig-4: Effect of different levels of pH on the mycelial growth of *M phaseolina*.**

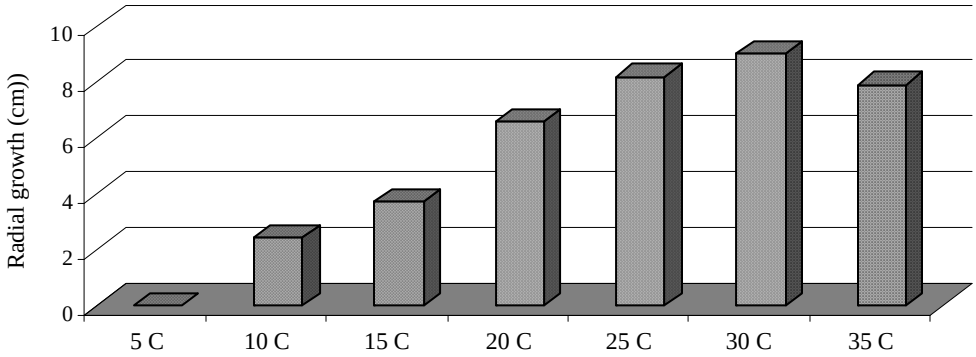


**Fig-5: Effect of the temperature ranges on the mycelial growth of *M. phaseolina*.**

## References

Anonymous, 2001. Agricultural Statistics of Pakistan. Ministry of Food, Agriculture and Livestock, Government of Pakistan, Islamabad.

Bais BS, Singh SB, Singh DV, 1970. Effect of different carbon and nitrogen sources on the growth and sporulation of *Curvularia pallescens*. *Indian Phytopath*, **23**: 511-517.

Chandra A, Purkayastha RP, 1977. Physiological studies on Indian edible mushrooms. *Trans Br. Mycol. Soc*,, **69**: 63-70.

Dhingra OB, Sinclair JB, 1978. Biology and pathology of *Macrophomina pahaeolina*. Univ. Fed de Viscose, Brazil 166 pp.

Hafeez A, Ahmad S, 2001. Screening of sunflower germplasm for resistance to charcoal rot. *Sarhad J. Agric*.,. **17**(4): 615-616.

Hoes JA, 1985. *Macrophomina phaseolina* causal agent of charcoal rot of sunflower and other crops. Agric. Canada Res. Stat. Modern Manitoba.

Mirza JH, Qureshi MSA, 1982. Fungi of Pakistan. Dept. Plant Pathology, Univ. Agric., Faisalabad, Pakistan. 311 pp.

Mirza MS, Beg A, Khan AR, 1982. Varietal screening of sunflower cultivars to charcoal rot caused by *Macrophomina phaseolina*. *Pakistan J. Agric. Res*., **3**(3): 202-203.

Mirza MS, Beg A, Asalm M, Ali N, 1986. Screening for resistance to *Macrophomina phaseolina* in sesame. *Pakistan J. Agric. Res*., **7**(1): 44-46.

Orellana RG, 1970. The response of sunflower genotypes to natural infection by *Macrophomina phaseolina*. *Plant Dis. Rep*., **54**: 891-893.

Shehzad S, Sattar A, Ghaffar A, 1988. Additions to the hosts of *Macrophomina phaseolina*. *Pak. J. Bot*., **20**: 151-152.

Sinclair JB, 1982. Compendium of Soybean diseases. 2nd ed. American Phytopathological Soc., St. Paul, MN. 104 pp.

Tikhonov OI, Nedelko VK, Perestora TA, 1976. Infection period and spreading pattern of *Sclerotium bataticola* in sunflower tissue. Proc. 7th Int. Sunflower Conf. p 134-135.