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# Studies on various factors influencing growth and pathogenicity of *Macrophomina phaseolina* in blackgram

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**Abstract**
Dry root rot of blackgram caused by *Macrophomina phaseolina* is the most devastating disease in all the urdbean growing districts of Tamil Nadu. An experiment was conducted to study the influence of temperature and pH on the growth of three isolates of *M. phaseolina* collected from major urdbean growing areas of Tuticorin district. Among the three isolates, *M. phaseolina* isolate 2 was the most virulent isolate. The pathogen grew well at 35 °C and no growth to very minimal growth was observed at 40 °C. The pathogen grew faster at pH 4 and 5 and gradually decreased at pH 6 and 7. Thus this experiment reveals that *M. phaseolina* grows well in acidic pH (4 and 5) and high temperature at 35 °C.

**Keywords:** Dry root rot, charcoal rot, temperature, pH

## Introduction
*M. phaseolina* is a necrotropic fungal pathogen attacking various crop plants including major food crops (maize, sorghum), pulse crops (blackgram, green gram, cowpea), oil seed crops (sesame, groundnut, sunflower, soybean) and fiber crops (cotton, jute). *M. phaseolina* causes many diseases such as dry root rot, seedling blight, charcoal rot and stem rot. Mostly, disease incidence is favored in summer season. Due to its strong scletorial persistence in soil, it is difficult to control *M. phaseolina* [1]. *M. phaseolina* is an anamorphic fungus in the *ascomycetes* class and *botryosphaeriaceae* family. The fungus does not have teleomorphic stage. The fungus survives by producing sclerotia that remain viable for more than four years in soil and crop residues. Under favorable environmental conditions, the fungal hyphae germinate from the sclerotia which infect the root of host plant resulting in yellowing of leaf followed by wilting which leads to plant death [1]. The disease incidence and development are favored under drought stress conditions. In this present study, we focus on isolation of various strains of *M. phaseolina* and to test the effect of major environmental factors such as temperature and pH that influence the growth of *M. phaseolina* and its pathogenicity in blackgram

## Materials and Methods
### Isolation of *M. phaseolina*
Infected black gram plants, showing typical root rot symptom were collected from various locations in Tuticorin district and used for the isolation of pathogen. The roots, collected from the infected plants, were thoroughly washed under running tap water to remove surface dust, soil and other contaminants. Infected tissues were cut into small pieces and surface sterilized with 2% sodium hypochloride for about three minutes followed by washing thrice using sterile water. The surface sterilized small pieces were plated on Potato Dextrose Agar Medium (PDA) and incubated for four days at 28 °C. Culture purification was carried out using hyphal tip method and the *Macrophomina* cultures collected from various locations were maintained separately on PDA slants by storing under refrigeration.

### Assessing the morphological characters of *Macrophomina* isolates
From the actively growing culture plates, nine milli metre culture disk was cut using sterilized cork borer and placed at the centre of PDA medium and incubated at room temperature (28±2 C) until the mycelial growth reached the edge of the Petri plates. The growth pattern and morphological characters of the isolates such as mycelial growth pattern, colony colour and colony character were recorded.

**Assessing the Pathogenicity Test**

Sand maize medium (5%) was prepared by mixing maize powder - 100 g and sieved river sand – 1900 g); moistened at 60 % and autoclaved in spawn covers at 20 lb. for two hours. Three different isolates of *M. phaseolina* were inoculated in sand maize medium and incubated at room temperature for 14 days for multiplication. This sand maize culture was mixed with pot mixture at five percent level and transferred to earthen pots. Pots with uninoculated soil served as control. Each pot was sown with seven healthy black gram seeds (variety KKM-1) and replicated four times. They were maintained in glass house. The plants exhibited root rot symptom around 25-35 days after sowing and the pathogen was re-isolated from these artificially inoculated plants and maintained on PDA slants for further studies. The per cent disease incidence was calculated as follows.

$$\text{Per cent disease incidence} \frac{\text{Number of diseased plants}}{\text{Total number of plants}} \text{ X } 100$$

**Assessing the growth of *M. phaseolina* at various temperatures**

Three different isolates of *M. phaseolina* was inoculated on the centre of Petri dish containing PDA medium and cultures were grown at four different temperatures *viz.*, 25 °C, 30 °C, 35 °C and 40 °C. Mycelial growth was recorded at 36 hrs, 48 hrs and 72 hrs. Each isolate was replicated five times.

**Assessing the growth of *M. phaseolina* at various pH**

PDA medium with four different pH levels *viz.*, 4, 5, 6 and 7 was prepared using phosphoric acid or sodium hydroxide. The media were poured in sterile Petri dishes. Nine milli metre culture disk of *M. phasiolina* isolates were cut using sterile cork borer and placed at the centre on PDA plates and incubated at 35 °C for 4 days. Mycelial growth were recorded when the growth of the mycelium covered in any one of the treatment.

**Results**

**Isolation of pathogen and its pathogenicity test**

Totally three isolates of *M. phaseolina* were isolated from infected black gram plants collected from Tuticorin district and the isolates were maintained on PDA slant at 4 °C for further studies. They were identified based on the production of microscopic black coloured irregular shaped sclerotia. *M. phaseolina* isolate 1 appeared as deep black with oblong sclerotia. Sclerotial production was noticed at four days after inoculation. *M. phaseolina* isolate 2 appeared as slight black colour and the sclerotial production was noticed at four days after inoculation. *M. phaseolina* isolate 3 appeared as whitish black colour with slight pluffy colony with oblong sclerotia. Sclerotial protection was noticed after 7-8 days after inoculation (Fig. 1; Table 1).

Pot culture experiment was conducted to test the pathogenicity of the *M. phaseolina* and results revealed that *M. phaseolina* isolate 1 recorded 42.66 per cent disease incidence. It exhibited brown to black colour lesion on stem at 23 days after sowing. *M. phaseolina* isolate 2 recorded 75.30 per cent disease incidence. It exhibited brown to black colour lesions on stem at 23 days after sowing. *M. phaseolina* isolate 3 recorded 40 percent disease incidence and exhibited brown to black colour lesion on stem at 30 days after sowing. To confirm the pathogenicity, the pathogen was re-isolated, and its characters were studied and compared with original culture. Among the three isolates tested, *M. phaseolina* isolate 2 was the most virulent culture. Lalita Lakhran *et al*. (2018) [6] also successfully proved the same pathogenicity test upon reisolation of virulent isolate of *M. phaseolina* resulted from their pot culture experiment.

**Effect of temperature on the mycelial growth of *M. phaseolina***

Three isolates of *M. phaseolina* were incubated at different temperatures *viz*., 25 °C, 30 °C, 35 °C and 40 °C and mycelial growth was measured at 36 hrs, 48 hrs and 72 hrs after inoculation. All three isolates grew well at 35 °C whereas very poor mycelial growth was observed at 40 °C and the sclerotial formation was good at 25 °C and 35 °C (Fig. 2; Table 2).

Temperature plays an important role in the growth of the pathogen and also in disease development. *M. phaseolina* grew well at high temperature. The present study revealed that high temperature at 35 °C favours the mycelial growth and disease development whereas very high temperature of 40 °C suppress the mycelial growth. Sukanya *et al*. (2016) [3] also concluded the same results i.e. the M. *phaseolina* grew faster at 35 °C and no mycelial growth was found at extremely high temperature of 45 °C.

**Effect of pH on the mycelial growth of *M. phaseolina***

To test the effect of pH on the mycelial growth, three isolates of *M. phaseolina* were inoculated on PDA medium with different pH levels *viz*., 4, 5, 6 and 7 and incubated at 35 °C for 4 days. Growth of all three isolates was maximum at acidic pH levels *viz.*, 4 and 5 and minimal growth was recorded in pH levels *viz.*, 6 and 7 which revealed that *M. phaseolina* grew faster in acidic pH than neutral and basic pH (Fig. 3; Table 3). Generally, fungi prefer slight acidic pH for their growth. Khamari *et al*. (2018) [4] found that maximum mycelial growth was observed at pH 6.5. However, the present study indicated that the pathogen grew well below the pH 6.

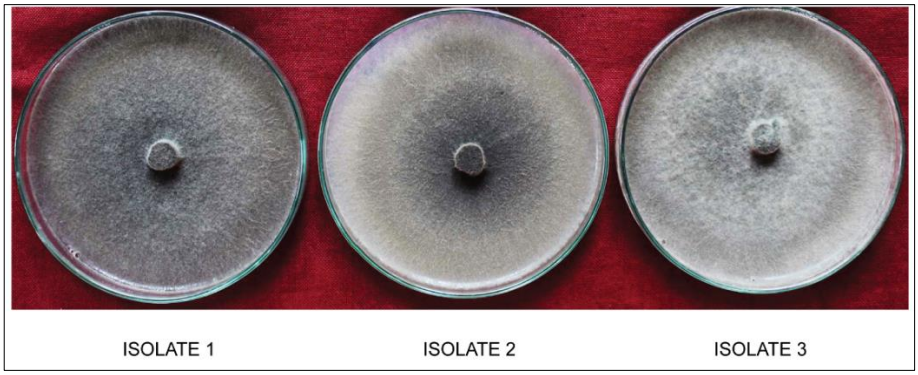


**Fig 1:** Cultural variability and pathogenicity of *M. phaseolina*

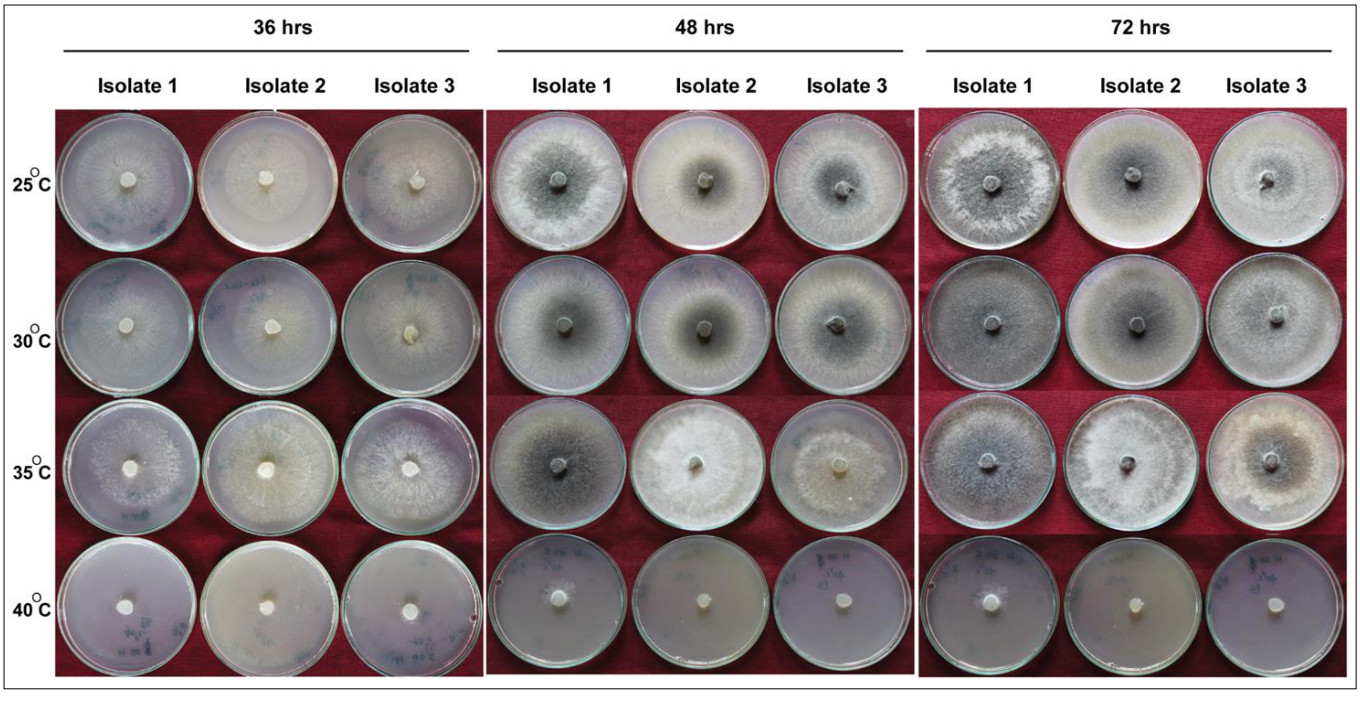


**Fig 2:** Effect of temperature on the mycelial growth of *M. phaseolina*

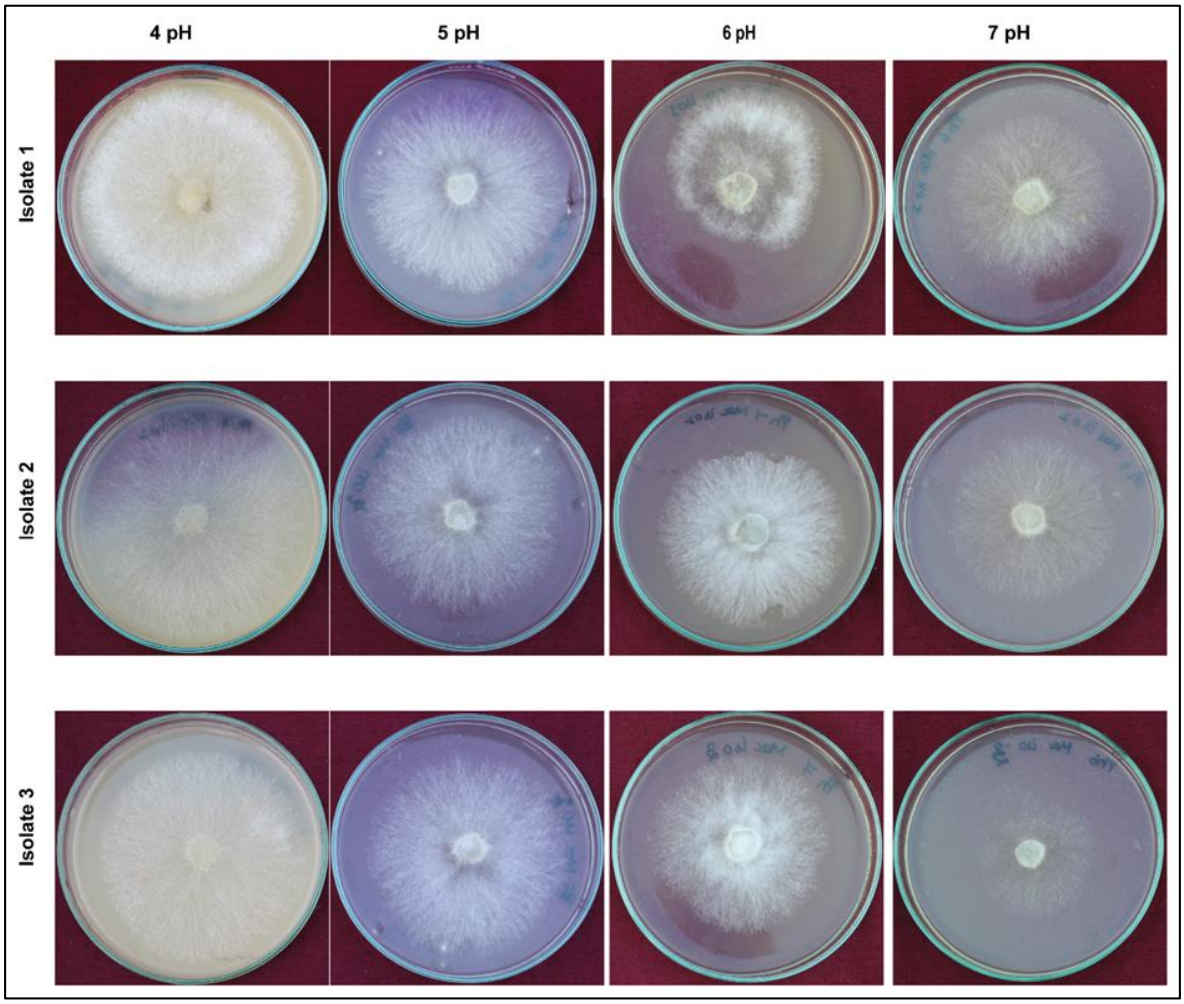


**Fig 3:** Effect of pH on the mycelial growth of *M. phaseolina*

**Table 1:** Pathogenicity of *Macrophomina phaseolina* with urd bean seeds

| S. No. | Inoculated isolates | Germination %* | Disease incidence %* |
|---|---|---|---|
| 1 | ISO-1 | 63.00[b] | 42.66[b] |
| 2 | ISO-2 | 58.4[c] | 75.30[a] |
| 3 | ISO-3 | 59.2[c] | 40.00[c] |
| 4 | Uninoculated control | 90.00[a] | 0.00[d] |
| CD Value | | 2.860 | 1.10 |

*Mean of four replications

**Table 2:** Effect of temperature on the mycelial growth of *M. phaseolina*

| S. No | Temperature | Mycelial growth (mm)* | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 48 hrs after inoculation | | | 60 hrs after inoculation | | | 84 hrs after inoculation | | |
| | | ISO-I | ISO-II | ISO-III | ISO-I | ISO-II | ISO-III | ISO-I | ISO-II | ISO-III |
| 1 | 25 °C | 47.6[ab] | 36.0[b] | 42.0[b] | 69.8[ab] | 56.0[b] | 64.0[b] | 90.0[a] | 90.0[a] | 90.0[a] |
| 2 | 30 °C | 44.4[b] | 44.4[a] | 47.0[a] | 67.2[b] | 61.6[b] | 65.0[b] | 90.0[a] | 90.0[a] | 90.0[a] |
| 3 | 35 °C | 52.3[a] | 50.0[a] | 48.4[a] | 72.0[a] | 73.2[a] | 69.6[a] | 90.0[a] | 90.0[a] | 90.0[a] |
| 4 | 40 °C | 4.3[c] | 0.5[c] | 0.50[c] | 13.2[c] | 6.2[c] | 9.60[c] | 17.2[b] | 10.8[b] | 10.8[b] |
| CD value(0.05) | | 5.16 | 6.34 | 2.48 | 3.93 | 7.38 | 3.540 | 2.858 | 0.75 | 0.38 |

*Mean of five replication

**Table 3:** Effect of pH on the mycelial growth of *M. phaseolina*

| S. No. | Isolates name | Mycelial growth after 72 hours of inoculation (mm)* | | |
|---|---|---|---|---|
| | | ISO-1 | ISO-2 | ISO-3 |
| 1 | PH 4 | 68.8[a] | 69.2[a] | 59.0[a] |
| 2 | PH 5 | 56.6[b] | 59.2[b] | 59.0[a] |
| 3 | PH 6 | 48.6[c] | 46.4[c] | 44.6[b] |
| 4 | PH 7 | 39.4[d] | 44.2[d] | 34.0[c] |
| CD(0.05) | | 2.109 | 1.496 | 3.11 |

*Mean of five replications

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