

Original Research Article

<https://doi.org/10.20546/ijcmas.2020.907.084>

Isolation and Evaluation of Various Isolates of *Macrophomina phaseolina* Causing Dry Root Rot of Sesame

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ABSTRACT

Keywords

Macrophomina phaseolina, Dry root rot, solid medium, liquid medium, pH

Article Info

Accepted:
08 June 2020
Available Online:
10 July 2020

Macrophomina phaseolina is an important pathogen in the sesame growing areas of Tamilnadu and is prevalent in the arid regions of the world. Studies were carried out to isolate *M. phaseolina* cultures and analyze their colony morphology, pathogenicity, and effect of culture media and pH conditions on the growth pattern was assessed. Among the five isolates, *M. phaseolina* isolate 4 was the most virulent isolate. The maximum growth of pathogen was observed in potato dextrose agar and potato dextrose broth. The pathogen grew well in neutral to slightly acidic conditions.

Introduction

Dry root rot caused by *Macrophomina phaseolina* (Tassi) Goid is the most serious pathogen affecting the crop at the later stages of growth causing up to 50 per cent disease incidence and resulting in heavy yield losses (Chattopadhyay and Kalpanasastry, 2002). The disease is both seed and soil-borne and usually infects the crop under dry and warm conditions. Sesame is mostly grown as a rainfed crop and under this situation, the crop is exposed to sufficient soil moisture during

its initial growth stages (up to 30-35 days), while subsequently the crop is maintained as a dry crop.

The dry condition prevalent during the later stage of the crop is a predisposing factor for infection of this root rot pathogen. *M. phaseolina* induces the symptoms such as infected seedlings shows reddish brown discoloration, failure of seed germination, browning and rotting of root, stem and seedlings which may ultimately leads to poor plant and finally very low yield.

Sclerotia (resting structure) survive on crop residues, dispersed by soil, contaminated seeds and farm equipments (Thiyagu *et al.*, 2007). In this study, the experiments were conducted to isolate various *M. phaseolina* isolates and to test their pathogenicity, the effect of various media and pH influencing the growth of *M. phaseolina*.

Materials and Methods

Isolation of pathogen

The disease infected (dark brown colour) stems of sesame plants were collected from the agricultural fields of Tuticorin district, Tamilnadu for the isolation of *M. phaseolina* causing dry root rot.

The collected stems were washed thoroughly under running tap water to remove surface dust, soil and other contaminants and surface sterilized with sodium hypo chloride. The upper layer of stem was scrapped, sliced as small pieces (~1 cm) and placed over the potato dextrose agar (PDA) in Petri plate and incubated at $28 \pm 2^\circ\text{C}$ for 4 days. The uniform growth of fungal mycelia around stem pieces was observed within 4 days and it was sub cultured for further use. *Macrophomina* cultures collected from various locations were maintained separately on PDA slants by storing under refrigeration.

Assessing the morphological characters of *M. phaseolina* isolates

Ninety millimetre of Petri dishes was poured with sterilized PDA medium and inoculated with actively growing cultures of *M. phaseolina* for assessing the morphological characters. The plates were incubated at room temperature ($28 \pm 2^\circ\text{C}$). Differences in morphological characters such as type of margin, growth pattern, colony colour and character was recorded.

Assessing the pathogenicity test for *M. phaseolina* isolates

Soil infestation method was followed to prove the pathogenicity of the fungus. The pathogen was mass multiplied on sand maize medium. Sand and ground maize grains were mixed in the ratio of 19:1, moistened to 50% and filled in spawn cover and autoclaved at 20 psi for 2h. Five *Macrophomina* isolates were inoculated in the sand maize medium under aseptic conditions and the medium was incubated at room temp. ($28 \pm 2^\circ\text{C}$) for 15 days. Pots of uniform size were used for proving pathogenicity. The inoculum of *M. phaseolina* isolates multiplied in sand maize medium was mixed with soil @ 5 % level ratio at the time of sowing.

Ten sesame seeds (variety VRI 2) were sown in each pot and maintained in green house with need based irrigation. The un-inoculated pots served as control. The PDI was assessed at 30, 60 and 90 DAS and recorded. Also the plants showing the typical root rot symptom were pulled out and the pathogen was re-isolated on PDA slants. The culture thus obtained was compared with that of the original culture and the pathogenicity (Koch postulates) was proved. The per cent disease incidence was worked out using the following formula

Per cent Disease Incidence (PDI)

$$= \frac{\text{No of diseased plants}}{\text{No of plants observed}}$$

Assessing the growth of *M. phaseolina* on different solid media

The mycelial growth of the fungus was assessed on various solid media: potato-dextrose agar (PDA), corn meal agar, oat meal agar and host specific medium (sesame). *M. phaseolina* cultures were cut using sterile cork borer and placed at the centre of Petridish and incubated at 35°C for

4 days. Mycelial growth was recorded when the growth of the mycelium covered in any one of the treatment.

Assessing the growth of *M. phaseolina* on different liquid media

Mycelial disc (9 mm) of *M. phaseolina* was taken from 3-4 days old culture and placed into each conical flask containing sterilized potato dextrose broth, corn meal broth, oat meal broth and host specific broth (sesame) and incubated at room temperature ($28 \pm 2^\circ\text{C}$) for seven days. After seven days the mycelial mat was filtered through Whatman No.1 filter paper and oven dried at 60°C for 48 h and weighed immediately. The conical flask containing potato dextrose broth was used as control. The dry weight of mycelium was recorded.

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

PDB with six different pH levels viz., 3, 4, 5, 6, 7 and 8 was prepared using phosphoric acid or sodium hydroxide. Nine millimetre culture disc of *M. phaseolina* isolates were cut using sterile corkborer and transferred into conical flasks containing 100 ml of medium at different pH levels and incubated at 35°C for 4 days. Mycelial growth was recorded when the growth of the mycelium covered in any one of the treatment.

Results and Discussion

Isolation of the pathogen

Totally five isolates of *M. phaseolina* were isolated from infected sesame 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 slightly whitish black colour with slight pluffy colony formed from hyphal aggregates with round /circular sclerotia. *M. phaseolina* isolate 2 appeared dark black colour with oblong sclerotia having irregular edges and sclerotial production was noticed at four days after inoculation.

M. phaseolina isolate 3 appeared greyish black colour with oblong to irregular shaped sclerotia. *M. phaseolina* isolate 4 appeared as greyish black colour with cottony white centre, differing in microsclerotial size and sclerotia are irregular to oblong shape. *M. phaseolina* isolate 5 appeared as jet black colour having sclerotia with regular edges. Sclerotial production was noticed after 6-7 days after inoculation (Figure 1).

Soil infestation method was followed to conduct the pathogenicity test of five isolates of *M. phaseolina* and results revealed that *M. phaseolina* isolate 1 recorded 41.90 per cent disease incidence. *M. phaseolina* isolate 2 recorded 47.62 per cent disease incidence. It exhibited black lesion on stem at 45 days. *M. phaseolina* isolate 3 recorded 50 per cent disease incidence. It exhibited brown to black lesion on stem. *M. phaseolina* isolate 4 recorded 73.80 per cent disease incidence. It exhibited brown to black brown lesion on stem at 45 days during flowering stage. At the later stages, complete drying or wilting of the plants were seen. *M. phaseolina* isolate 5 recorded 50 per cent disease incidence. It exhibited black to brown lesion but showed high germination percentage. The pathogen was re-isolated, and its characters were recorded for comparison with original culture for the conformation of pathogenicity test. Among the five isolates tested, *M. phaseolina* isolate 4 was the most virulent culture (Figure 2; Table 1). Akhtar *et al.*, (2011) reported the pathogenicity test at seedling stage and recorded the virulence of the pathogen.

Table.1 Pathogenicity test of *Macrophomina phaseolina*

S.No.	Inoculated isolates	Germination%*	Disease incidence%*
1	ISO-1	63.33 ^b	41.90(40.33) ^c
2	ISO-2	70.00 ^b	47.62(43.60) ^{bc}
3	ISO-3	73.33 ^b	50.00(45.00) ^b
4	ISO-4	76.66 ^b	73.81(59.22) ^a
5	ISO-5	73.33 ^b	50.00(44.99) ^b
6	Un inoculated control	93.33 ^a	0.00(0.00) ^d
CD(0.05)		15.86	3.64

*Mean of three replications

The values in the parentheses are arc sine transformed values.

The treatment means are compared using Duncan Multiple Range Test (DMRT)

Table.2 Effect of different solid media on the mycelial growth of *M.phaseolina*

Media	Mycelial growth (mm)*
PDA	82.00 ^a
Host specific medium (sesame)	75.33 ^b
Corn meal agar	62.33 ^c
Oat meal agar	55.00 ^d
CD(0.05)	3.58

*Mean of four replications

The treatment means are compared using Duncan Multiple Range Test (DMRT)

Table.3 Effect of different liquid media on the mycelial growth of *M.phaseolina*

Media	Mycelial dry weight (g)*
PDB	1.08 ^a
Host specific medium(sesame)	0.40 ^b
Corn meal broth	0.17 ^c
Oat meal broth	0.15 ^d
CD(0.05)	0.10

*Mean of three replications

The treatment means are compared using Duncan Multiple Range Test (DMRT)

Table.4 Effect of pH on the mycelial growth of *M.phaseolina* on PDB

S.No.	Different pH	Myelial dry weight after 7 of inoculation(g)*
1	pH 3	0.40 ^b
2	pH 4	0.43 ^b
3	pH 5	0.55 ^a
4	pH 6	0.60 ^a
5	pH 7	0.57 ^a
6	pH 8	0.53 ^{ab}
CD value(0.05)		0.10

*Mean of three replications

The treatment means are compared using Duncan Multiple Range Test (DMRT)

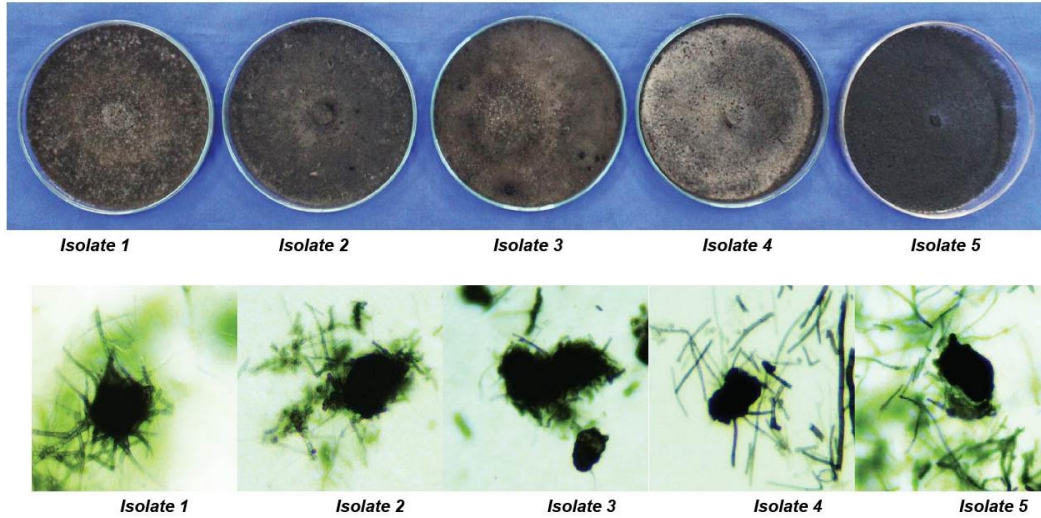


Fig.1 Cultural variability of *Macrophomina phaseolina* isolates and Sclerotial formation of *Macrophomina phaseolina* isolates

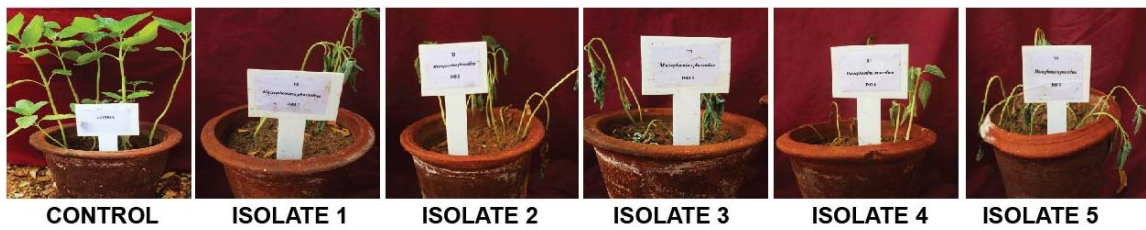


Fig.2 Pathogenicity test

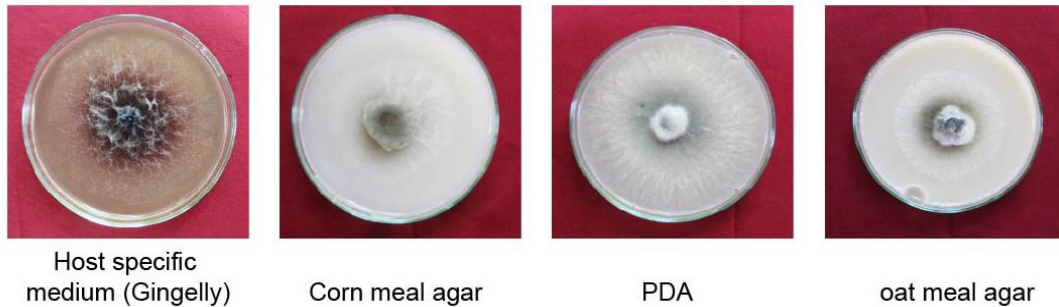


Fig.3 Effect of different solid media on mycelial growth of *Macrophomina phaseolina*

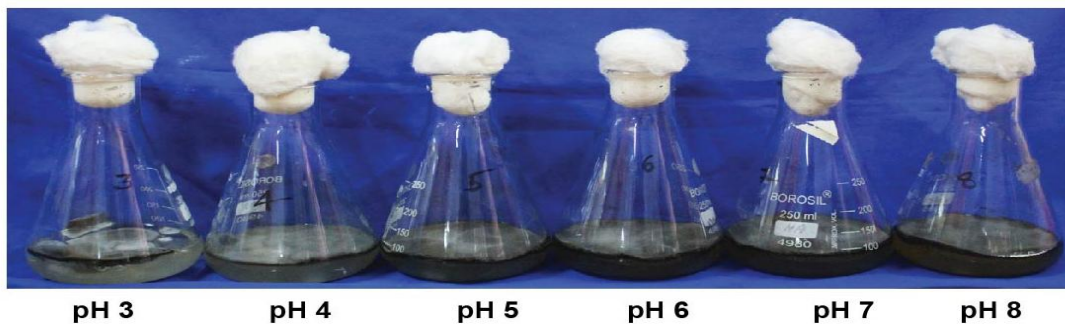


Fig.4 Effect of different pH on the mycelial growth of *Macrophomina phaseolina*

Effect of different solid and liquid media on the mycelial growth of *M.phaseolina*

Radial growth and mycelial dry weight of *M.phaseolina* isolate 4 on the four different solid and liquid media were assessed. The mean radial mycelial growth of the isolates on different solid media ranged between 82.00 mm and 55.00 mm.

Among the four solid media tested, PDA supported the highest mycelial growth of 82.00 mm followed by host specific medium 75.33 mm, corn meal agar 62.33 mm and oat meal agar 55.00 cm (Figure 3; Table 2).

Similarly, among the four liquid media tested, potato dextrose broth (PDB) yielded the maximum biomass of 1.08 followed by host specific broth 0.40 g, corn meal broth 0.17 g, oat meal broth 0.15 g.

Tandel *et al.*, (2012) found that potato dextrose agar is the best solid media and potato dextrose broth was the best liquid media for the mycelial growth and sclerotial formation of *M. phaseolina* (Table 3).

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

To test the effect of pH on the mycelial growth, *M.phaseolina* isolate 4 was inoculated in the PDB with different pH levels viz., 3,4,5,6, 7 and 8 and incubated at 35°C for 4-5 days.

From this experiment, it was noticed that *M.phaseolina* preferred to grow well at slightly acidic to neutral pH conditions (pH 6-7) (Figure 4; Table 4). Sukanya R *et al.*, (2016) found that maximum dry mycelial weight was at pH 6.0

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How to cite this article:

Premalatha. K., R. Oviya, R. Kannan, K. G. Sabarinathan and Ramamoorthy. V. 2020. Isolation and Evaluation of Various Isolates of *Macrophomina phaseolina* Causing Dry Root Rot of Sesame. *Int.J.Curr.Microbiol.App.Sci.* 9(07): 728-734.
doi: <https://doi.org/10.20546/ijcmas.2020.907.084>