Research Article

# Effect of Different Media, Temperature and pH on Growth and Microsclerotia Formation of *Macrophomina phaseolina* Causing Charcoal Rot of Soybean

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**Abstract**

Charcoal rot of soybean caused by *Macrophomina phaseolina* is one of the destructive diseases in soybean growing areas of the Madhya Pradesh. *In vitro* studies were conducted on the effect of culture media, temperature and pH levels on the mycelial growth and microsclerotia production of *M. phaseolina* causing Charcoal rot of soybean. Among the seven solid and liquid media tested, potato dextrose agar was the best among the solid media and Richard's broth was the best among liquid media for the mycelial growth and biomass production of *M. phaseolina*. The optimum pH range for maximum growth (90.0 mm) and sclerotia formation was from 6.0-7.0. The optimum temperature for growth of *M. phaseolina* was at 30°C which was reduced significantly below 25°C and above 35°C.

**Keywords:** Media, pH, Temperature, *Macrophomina phaseolina*, Charcoal rot, Soybean

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## Introduction

Soybean has become an important oilseed crop in India with an area and production of respectively, 113.98 lakh ha and 135.05 lakh tonnes during *kharif* 2019-20. The major soybean growing states are Madhya Pradesh, Maharashtra, Rajasthan, Karnataka, and Telangana. Madhya Pradesh contribution has always been largest and substantial in respect of area and production of country's total. Madhya Pradesh has 45 percent share in production. This fact has established Madhya Pradesh as synonym of Soya State. However, the productivity of soybean is much lower than the actual yield potential of the crop. The major constraints of low productivity of this crop are the heavy losses caused by diseases, pests and weeds. Soybean is susceptible to a large number of disease agents such as seedling and root pathogens that cause serious damages to this crop. One of these soil borne pathogens is *Macrophomina phaseolina* [1, 2] the causal agent of charcoal root rot. Various factors are responsible for low productivity of soybean among them diseases is major concerns. Charcoal rot of soybean caused by *Macrophomina phaseolina* [1, 2] is one of the most important soil borne pathogens, infecting over 500 plant species in more than 100 plant families around the world [3, 4].

In order to culture the fungi artificially, it is necessary to supply all essential nutrients needed for their growth and development. Similarly, suitable temperature is also important for growth and metabolic processes of fungi. Therefore, the experiments have been carried out to know the effects of culture media and temperature on mycelial growth and sclerotial formation of *M. phaseolina.*

## Material and Methods

Isolate of *Macrophomina phaseolina* was recovered from diseased soybean plants from research farm of Jwaharlal Nehru Krishi Vishwa Vidyalaya-Jabalpur. Small pieces of infected tissues 1–2 mm dimension from the advancing margin of the spot, adjacent to healthy portions were cut with blade, washed well in distilled water to remove dust adhered to the infected pieces. Pieces were dipped in 0.1 percent mercuric chloride solution for 30 seconds and finally washed well in three changes of sterilized distilled water. The bits were then transferred to PDA slants with the help of inoculating needle under aseptic condition and incubated at 28 ± 1ºC. After 48 hrs, fragments of hyphal growth from the growing tips were transferred to fresh PDA slants. Pure culture was made, following repeated hyphal tip transfer. Pure culture was maintained on PDA slants by sub culturing it at 30 days intervals. For preservation of cultures the plugged end of the culture tubes were dipped in melted wax and stored in a refrigerator at 5 ± 1ºC.

### *Effect of Solid and liquid media*

Seven media *i.e.,* potato dextrose agar, Czapek's dox agar, Richard's agar, Asthana and Hawker's agar, Browns agar,

Ashby's agar and Coon's agar medium were used. These agar based sterilized media were poured into 90 mm diameter sterilized Petri plates @ 20 ml/plate. After solidification, 5 mm diameter culture block of seven days old pure culture of *M. phaseolina* was cut with the help of sterilized cork borer and placed in the centre of petriplates. Three replications were kept for recording observations on colony diameter, sclerotial formation and colony character of the fungus. The petriplates were incubated at room temperature (28 ± 1ºC) and observations were recorded after four days of incubation.

All the solid media used in earlier section were used as broth media with the same ingredients. Similarly, 50 ml of liquid media prepared in 150 ml conical flask for each medium with four replications. Sterilized media containing flask were incubated with test fungus. Out of four replications, one replication was kept for counting sclerotial formation of fungus. The rest of the replications were kept for recording dry mycelium weight of the fungus, which were incubated at room temperature for a period of 15 days. After incubation period, the mycelial mats were filtered through Whatman filter paper and the filter papers with mycelial mats were dried in an oven at 60ºC for 24 hours and dry weight of the mycelium was recorded till constant weight. Sclerotia count was recorded from fourth replication. The whole mycelial substrate was homogenized in 50 ml sterilized distilled water with the help of homogenizer and substrate was filtered through muslin cloth. A drop of the filter was examined and the numbers of sclerotia were counted. Sclerotial formations were counted in fungal culture suspensions under the microscope at low power (10x). The fungal culture suspension was prepared by vigorously shaking the 4 mm mycelial disc of the fungus in 10 ml sterilized distilled water. The sclerotial count was grouped as: - = absent; + = 1-4; ++ = 5-8; +++ = 9-15 and ++++ = above 10.

### *Effect of pH*

The growth of *M. phaseolina* was tested at 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, and 8.5 pH with a difference of 0.5 were prepared by using pH meter and by using either N/10 HCl or NaOH before autoclaving the PDA medium. Sterilized potato dextrose agar was poured into 90 mm diameter sterilized Petri plates. After solidification, 5 mm disc from actively growing cultures were cut and inoculated to solidified petriplates and incubated for 4 days in the incubator adjusted to required temperature levels at 28 $\pm$ 1ºC. Each treatment was replicated thrice. After incubation period, radial growth and sclerotial formation from solid media were recorded.

### *Effect of temperature*

The growth of *M. phaseolina* was tested at 10, 15, 20, 25, 30, 35 and 40 ºC. Sterilized potato dextrose agar was poured into 90 mm diameter sterilized petriplates. After solidification, 5 mm disc from actively growing cultures were cut and inoculated to solidified petriplates and incubated for 4 days in the incubator adjusted to required temperature levels at 28 ± 1ºC. Each treatment was replicated thrice. After incubation period, radial growth and sclerotial formation from solid media were recorded.

## Results and Discussion

The result presented in **Table 1** revealed that among all solid media tested, maximum mycelial growth was obtained in Potato dextrose agar (90 mm), which was statically at par with Richard's agar (89.0 mm). The rest of the media *viz.,* Czapek's dox agar (87.0 mm), Asthana and Hawker's agar (85.66 mm), Coon's agar (84.0 mm), Browns agar (47.0 mm), and Ashby's agar (46.33 mm) were also supported good growth of the fungus *M. phaseolina.* Good sclerotial formation was observed in potato dextrose agar and Richard's agar medium.

In liquid media, maximum dry mycelial weight was recorded in Richard's agar (776.25 mg) which was significantly superior over rest of the broth media (Table 1). In order of merit, the next best media was potato dextrose agar (758.56 mg) followed by Czapek's dox agar (602.0 mg), Asthana and Hawker's agar (263.03 mg), Coon's agar (180.19 mg) and Ashby's agar (134.89 mg) Browns agar (102.33 mg). While, Browns agar (102.33 mg) were poor as it yield less dry mycelial yield. Abundant sclerotial formation was observed in potato dextrose agar and Richard's agar in both solid and liquid medium. These results are in confirmation with [5-9].

Growth and sclerotia formation of the test fungus was observed at all the pH levels tested but it was maximum at pH range from 6.0-7.0 (90.0 mm) after 4 days of incubation (**Table 2**). The results of the present study are in agreement with [10, 11].

Temperature plays an important role in infection and disease development. Data presented in **Table 2** revealed that, maximum mean radial growth of test fungus was recorded at temperature of 30°C (87.56 mm) which was significantly superior over all other temperature. Lowest mean colony diameter was obtained at temperatures of 10°C (20.46 mm) and 40°C (26.65 mm). In the present study, it was observed that temperature range of 25°C to 30°C can

be recommended to obtain excellent fungal growth and sclerotial formation of *M. phaseolina.* These results were in confirmation with [12] found most favourable temperature range was 25 to 35 $^{0}$C for *M. phaseolina* isolates and mycelial growth of pathogen was very low at 10, 15 and 40°C and did not form sclerotia. **[**8] found most favorable temperature range between 25 to 35°C for mycelial growth and sclerotial formation of *M. phaseolina*.

**Table 1** Effect of solid and liquid media on radial growth and microsclerotia formation of *Macrophomina phaseolina*

| S.No | Name of the medium | Solid Media | | Liquid Media | |
|---|---|---|---|---|---|
| | | Colony diameter (mm) after 96 hrs* | Microsclerotia formation | Dry mycelial weight (mg) after 21 days* | Microsclerotia formation |
| 1 | Potato dextrose agar | 90.00 | +++ | 758.56 | +++ |
| 2 | Richard's agar | 89.00 | +++ | 776.25 | +++ |
| 3 | Czapek's Dox agar | 87.00 | ++ | 602.00 | ++ |
| 4 | Asthana and Hawker's agar | 85. 66 | ++ | 263.03 | ++ |
| 5 | Coon's agar | 84.00 | ++ | 180.19 | + |
| 6 | Browns agar | 47.00 | + | 102.33 | ++ |
| 7 | Ashby's agar | 46.33 | + | 134.89 | + |
| | CD (0.05) | 1.060 | | 1.867 | |

*Average of 3 replications

**Table 2** Effect of various pH and temperature on radial growth and microsclerotia formation of *Macrophomina phaseolina*

| S. No. | pH | Colony diameter (mm) after 96 hrs* | Degree of microsclerotia formed after 21 days* | Temperature (°C) | Colony diameter (mm) after 96 hrs* | Degree of microsclerotia formed after 21 days* |
|---|---|---|---|---|---|---|
| 1 | 5.0 | 80.0 | ++ | 10 | 20.4 | - |
| 2 | 5.5 | 84.0 | +++ | 15 | 27.8 | ++ |
| 3 | 6.0 | 90.0 | ++++ | 20 | 52.9 | +++ |
| 4 | 6.5 | 90.0 | ++++ | 25 | 80.1 | +++ |
| 5 | 7.0 | 90.0 | ++++ | 30 | 87.5 | ++++ |
| 6 | 7.5 | 77.3 | +++ | 35 | 71.8 | ++ |
| 7 | 8.0 | 28.66 | + | 40 | 26.6 | - |
| 8 | 8.5 | 29.00 | Poor | | | |
| CD (0.05) | | 1.891 | | | 2.341 | |

*Average of 3 replications

## References


[1] Tassi A. 1901. Novae micromycetum species. Ser III Bull Lab Orto Bot Univ Siena. 4:7–12.

[2] Goidanich G., 1947. A Revision of the genus Macrophomina Petrak. type species: M. P. (Tassi) G. Goid. n. comb. M. P. (Maubl.) Ashby. Ann Sper Agr N S 3:449–461.

[3] Smith, G. S., Wyllie, T. D., Hartman, L., Sinclair, J. B., Rupe, J. C., (Eds.). 1999. Compendium of Soybean Disease (fourth ed.), American Phytopathological Society, St. Paul, MN. Pp. 29-31.

[4] Vibha, 2016. *Macrophomina phaseolina*: The Most Destructive Soybean Fungal Pathogen of Global Concern. In: Kumar P., Gupta V., Tiwari A., Kamle M. (eds) Current Trends in Plant Disease Diagnostics and Management Practices. Fungal Biology. Springer, Cham. Pp. 193-205.

[5] Jha, A., K., and Dubey, G. K. 2000. Evaluation of fungicides for the control of charcoal rot of soybean caused by *Macrophomina phaseolina*. Indian Phytopathology. (2): 21-23.

[6] Tandel, D. H., Sabalpara, A. N., and Patel, R. C. 2012. Evaluation of different solid and liquid media for the growth and sclerotial formation of *Macrophomina phaseolina* Goid *in vitro*. The Bioscan. 7(4): 743- 745.

[7] Chaudhary, B., Kumar, S., Sharma, R. L., and Jakhar, S. R. 2018. Effect of Different Media, pH and Temperature on Growth and Sporulation of *Fusarium udum* Causing Wilt of Pigeonpea. International Journal of Current Microbiology and Applied Sciences. Special Issue-6: 2005-2011.

[8] Parmar, H., Kapadiya, H. J., Bhaliya, C. M., and Patel, R. C. 2018. Effect of Media and Temperature on the Growth and Sclerotial Formation of *Macrophomina phaseolina* (Tassi) Goid causing Root Rot of Castor. International Journal of Current Microbiology and Applied Sciences. 7(2): 671-675.

[9] Kushwaha, S. K., Kumar, S., Chaudhary, B., and Sahu, R. 2019. Effect of Different Media, pH and Temperature on Growth and Sclerotia Formation of *Sclerotium rolfsii* Sacc. causing Collar rot of Lentil. Chemical Science Review and Letters. 8(29), 01-05.
[10] Nischwitz, C., Olsen, M., and Rasmussen, S. 2004. Effect of irrigation type on inoculums density of *Macrophomina phaseolina* in melon fields in Arizona. J Phytopathol. 152:133–137.
[11] Kulkarni, S. 2000. Biology and management of dry stalk rots of maize (Zea mays L.) caused by *Fusarium moniliformae* Sheild and *Macrophomina phaseolina* (Tassi.) Goid. (Unpublished doctoral thesis), University of Agricultural Sciences, Dharwad, Pp. 160-164.
[12] Csondes, L., Kadlicsko, S., and Gaborjanyi, R. 2007. Effect of different temperature and culture media on the growth of *Macrophomina phaseolina*. Bio Sci., 72(4): 839-848.

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