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CULTURAL AND PHYSIOLOGICAL STUDIES AGAINST *MACROPHOMINA PHASEOLINA* INCITING DRY ROOT ROT DISEASE IN SOYBEAN

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ABSTRACT

Soybean (*Glycine max* L.) is one of the most economically important crops in the world. *Macrophomina phaseolina*, is a soil-borne fungal pathogen causing dry root rot disease in soybean is responsible for causing significant yield losses of the crop. The present study focused on physiological and cultural studies. Among the nine-culture media evaluated, Potato Dextrose Agar supported the highest mycelial growth and was identified as the most suitable medium for culturing *M. phaseolina*. Optimal mycelial growth was observed at 30°C and pH 7.

Key words : Soybean, Dry root rot, *Macrophomina phaseolina*, Culture media, Temperature, pH.

Introduction

The cultivation of soybean can be traced back to approximately 5,000 years ago in China (Liu *et al*., 2019). It is globally valued for its relatively high-quality oil and protein content, which constitute approximately 20% and 40% of the soybean, respectively (Clemente and Cahoon, 2009).

Currently, soybean cultivation in India is predominantly confined to the states of Madhya Pradesh, Maharashtra, Uttar Pradesh, Gujarat, Rajasthan, Himachal Pradesh, Telangana and Uttarakhand. (Reddy *et al*., 2019). During the year 2024–25, the total area under soybean cultivation in India was 12.93 million hectares, with a production of 13.36 million tonnes and a productivity of 1,033 kg/ha (Anonymous, 2025).

Globally, dry root rot (also referred to as charcoal rot or crown root rot), caused by *Macrophomina phaseolina*, is a significant disease impacting a wide range of plantation, arable, and horticultural crops, including cereals and legumes (Iqbal and Mukhtar, 2014). Among leguminous crops, dry root rot leads to considerable yield losses in both chickpea and soybean (Gupta *et al*., 2012a; Sharma *et al*., 2012). The pathogen, *M. phaseolina* is characterized by hyaline, thin-walled hyphae that may become light to dark brown and septate with age. Lateral branches typically emerge at right angles from the main hyphae and exhibit a constriction at the point of origin. Microsclerotia, which are compact masses of hardened mycelium, appear spherical, oval, or oblong, initially light brown and progressively turning dark brown to black as they mature. Pycnidia, which are infrequently observed under natural conditions are larger than microsclerotia, dark brown to black, rough in texture, globose or irregular in shape, beaked and ostiolated (Lakhran *et al*., 2018).

The disease is more severe in regions where the climate is relatively dry and warm during the growing season (Singh and Mehrotra, 1982). This necrotrophic fungus can persist in the soil in a viable state for several years. Under conditions of elevated temperatures (30–35°C) and low soil moisture levels (below 60%), it can lead to substantial yield losses in crops such as soybean and sorghum, adversely affecting farmer's incomes. (Kaur *et al*., 2012).

Materials and Methods

Isolation and Identification of Pathogen

The pathogen was isolated from the diseased root tissue of plant samples collected from a farmer's field.

Identification of the pathogen was carried out based on its morphological and cultural characteristics, including spore morphology and colony features. For microscopic examination, a sporulating culture of the test pathogen was mounted on a glass slide using lactophenol cotton blue stain and observed under a research microscope at 400× magnification. Pathogenicity was confirmed using the sick soil method.

Cultural and physiological characteristics of *M. phaseolina*

Effect of different culture media on *M. phaseolina*

The culture media *viz.*, Potato dextrose agar, Richard's agar, Koon's agar, Malt extract agar, Nutrient agar, Corn meal, Czepk's, Oat meal agar, Sabouraud's, Corn meal media were used to study the cultural characteristics of *Macrophomina phaseolina*. A disc of 7 days old culture (5 mm) of test fungus was placed in the centre of previously poured and solidified media (20ml) in glass petriplates (90mm) at 27±1. Four replications were maintained Observation on colony growth, colony characters and sporulation were recorded after seven days of incubation.

Effect of temperature on growth of *Macrophomina phaseolina*

The effect of temperature on the growth of *M. phaseolina* on PDA media was studied by incubating cultures at six different temperatures *viz.*, 10, 15, 20, 25, 30 and 35°C with three replications. The extent of colony growth was measured after seven days.

Effect of different pH levels on growth of *Macrophomina phaseolina*

The studies were conducted by using PDA as medium for growth of the pathogen, pH of the medium was adjusted before autoclaving using 0.1N HCL and 0.1N NaOH, measured with a digital pH meter. After sterilization, the medium was poured into sterile Petri plates in three replicates. Each plate with pH values of 5.5, 6.0, 6.5, 7.0, 7.5, 8.0 and 8.5 were inoculated with seven days old 5mm of *M. phaseolina*.

Results and Discussion

Colony characteristics of *M. phaseolina* on different cultural media

From the data presented in Table 1, it is revealed that among various culture media under study, PDA strongly supported the growth of the pathogen and highest colony diameter (90.00mm) was recorded on it. This was closely followed by Richard's Agar (87.00 mm), Nutrient Agar (85.00 mm), Koon's Agar (82.0 mm), Corn meal Agar (80.33mm), Oat meal Agar (77.16mm), Malt extract Agar (75.00mm) and Czepk's Agar (65.23mm). In contrast, Sabouraud's Agar exhibited lowest colony diameter (45.00mm). These observations are consistent with findings by Kumar and Chaudhary (2020), who

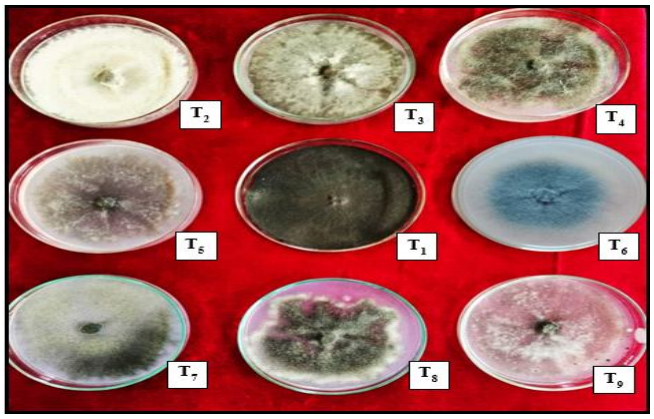

Plate 1 : Effect of different culture media on growth of *Macrophomina phaseolina*.

Table 1 : Effect of different culture media on growth of *Macrophomina phaseolina.*

S. no.	Culture media	MCD (mm)*	Colony characters	Sporulation (Grade)
1.	Potato Dextrose Agar	90.00	White in center and periphery deep black, circular colony	++++
2.	Koon's Agar	84.50	White in center and periphery whitish, circular colony	+++
3.	Richard's Agar	87.00	White in center and periphery black, circular colony	++++
4.	Oat meal Agar	77.16	Black in centre and periphery brown, dense mycelial mat	++
5.	Malt Extract Agar	75.00	White in center and periphery faint black, circular colony	++
6.	Sabouraud'sAgar	45.00	Faint black in center and periphery white, circular colony	+
7.	Nutrient Agar	85.00	White in center and periphery deep black, circular colony	+++
8.	Corn meal Agar	80.33	Black in center and periphery dark white, thin mycelial mat	+++
9.	Czepk's Agar	65.23	White in center and periphery whitish, circular colony	++
S.E. (±m)		**0.35**		
C.D.@1%		**1.01**		

*= Mean of four replications.

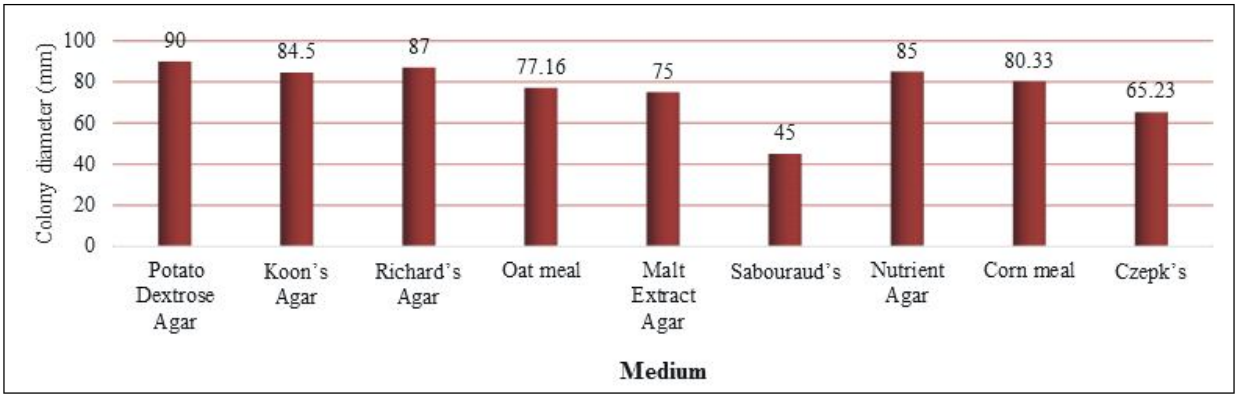

Fig. 1 : Effect of different culture media on growth of *Macrophomina phaseolina.*

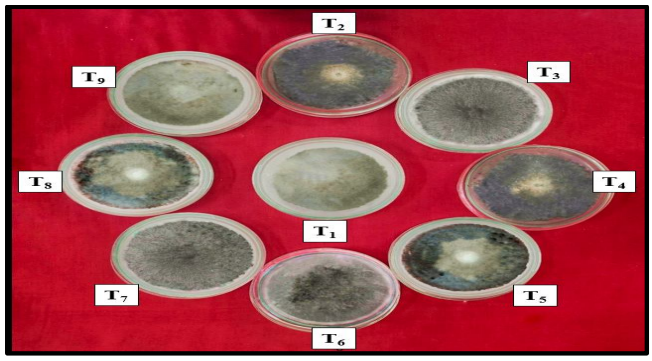

Plate 2 : Effect of pH level on growth of *Macrophomina phaseolina.*

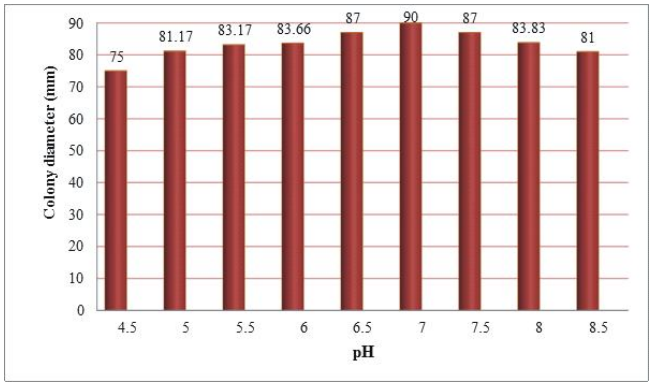

Fig. 2 : Effect of pH level on growth of *Macrophomina phaseolina*.

Table 2 : Effect of temperature on growth of *Macrophomina phaseolina*.

Tr. no.	Temperature (°C)	MCD (mm)*	Sporulation (Grade)
T_1	10	7.00	-
T_2	15	22.00	+
T_3	20	53.50	++
T_4	25	80.00	+++
T_5	30	90.00	++++
T_6	35	88.50	++++
S.E. (±m)		**0.60**	-
C.D.@1%		**1.85**	-

* = Mean of four replications.

reported maximum growth of *M. phaseolina* on PDA.

Sclerotial production was abundant (++++) on both PDA and Richard's Agar. Koon's Agar, Nutrient Agar while Corn meal Agar supported good (+++) sclerotial formation. Whereas Malt Extract Agar, Oat meal Agar and Czepk's Agar resulted in moderate (++) sclerotial development. Sabouraud's Agar, however formed scanty (+) sclerotial bodies. This result are in agreement with previous workers; Salunkhe (2009), Nagamma *et al.* (2015), Gaikwad and Rajurkar (2018), who also reported maximum sclerotial production on PDA.

Growth of *M. phaseolina* at various temperature

Among the tested temperatures, maximum radial growth was observed at 30°C, with a colony diameter of 90.00 mm, closely followed by 35°C (88.50 mm) and 25°C (80.00 mm). Moderate growth was noted at 20°C (53.50 mm), while a significant reduction in growth occurred at 15°C (22.00 mm). At 10°C, the pathogen showed minimal development, with colony diameters of 7.00 mm in Table 2. Sukanya *et al.* (2016) reported that the radial growth of the pathogen was maximal (90 mm) at 30°C and 35°C. Sclerotial production by *M. phaseolina* was found to be abundant (++++) at 30°C and 35°C, good (+++) at 25°C, moderate (++) at 20°C, and scanty (+) at 15°C. No sclerotia were produced at 10°C. Similarly, Kaur *et al.* (2012) reported that 30°C is the most favorable temperature for maximal sclerotial production in *M. phaseolina.*

Growth of *M. phaseolina* at various pH levels

The data is presented in Table-3 revealed that *Macrophomina phaseolina* exhibited maximal mycelial growth at pH 7.0 (90.00 mm), followed closely by pH 7.5 (87.00 mm), pH 6.5 (87.00 mm), pH 8.0 (83.83 mm) and pH 6.0 (83.66 mm). A progressive decline in growth

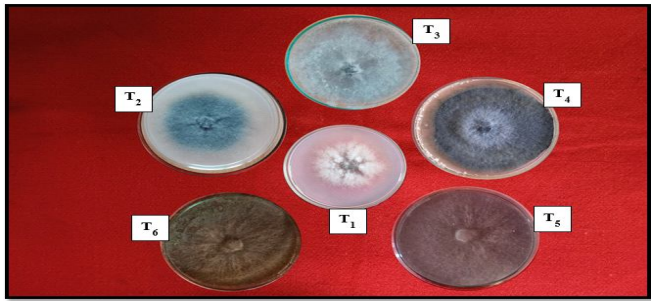

Plate 3 : Effect of temperature on growth of *Macrophomina phaseolina*.

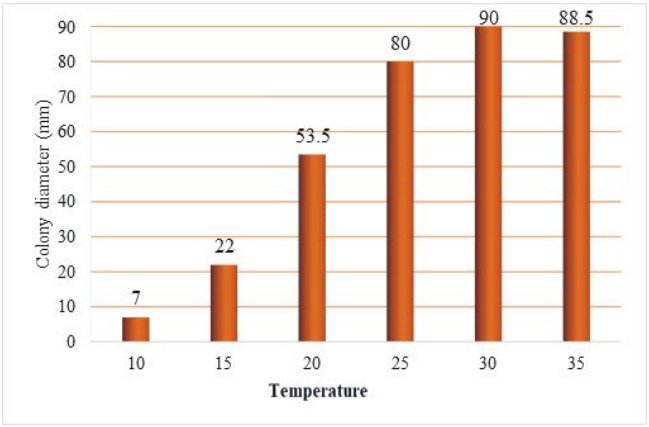

Fig. 3 : Effect of temperature on growth of *Macrophomina phaseol*.

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

Tr. no.	pH	MCD (mm)*	Sporulation (Grade)
T_1	4.5	75.00	++
T_2	5.0	81.17	+++
T_3	5.5	83.17	+++
T_4	6.0	83.66	+++
T_5	6.5	87.00	++++
T_6	7.0	90.00	++++
T_7	7.5	87.00	++++
T_8	8.0	83.83	+++
T_9	8.5	81.00	+++
S.E. (±m)		**0.94**	-
C.D.@1%		**2.81**	-

*= Mean of four replications.

was observed at more acidic and alkaline conditions, with reduced mycelial growth at pH 5.5 (83.17 mm), pH 8.5 (81.00 mm), and pH 5.0 (81.17 mm). The minimum growth was recorded at pH 4.5 (75.00 mm).

Regarding sclerotial production, *M. phaseolina* showed abundant production (++++) at pH 6.5, 7.0 and 7.5. Good production (+++) was observed at pH 5.0, 5.5, 6.0, 8.0, and 8.5, while moderate production (++) occurred at pH 4.5. Previously, Bhupathi and Theradimani (2018) identified pH 7.0 as the most conducive for both mycelial growth and sclerotial development in black gram. Furthermore, Kumar and Choudary (2020) concluded that the pH range of 6.0–7.0 is optimal for both mycelial growth (90.0 mm) and sclerotial development.

Conclusion

Thus, from the results, it is concluded that PDA was identified as the most conducive substrate for vegetative growth and sclerotial development of *M. phaseolina* isolates, followed by Richard's Agar media. *M. phaseolina* displays variable growth responses across different pH levels, with an optimal growth range between pH 6.5 and 7.5 and also 30°C to 35°C as optimal for the vegetative growth.

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