



ISSN Print: 2664-844X
 ISSN Online: 2664-8458
 NAAS Rating (2026): 4.97
 IJAFS 2026; 8(9): 694-698
www.agriculturaljournals.com
 Received: 03-07-2026
 Accepted: 06-08-2026
 Published: 22-09-2026

The names and affiliations of all authors are provided below, following the references.

Effect of different culture media on the growth and sporulation of *Macrophomina phaseolina* causing dry root rot of pigeonpea

Aditi Rajesh Joshi¹, SV Kolase², SB Latake³, VS Shinde⁴, SA Raut⁵, DH Sarnobat⁶,
 Vaibhavi Vitthalrao Ganjare⁷, Snehal Sudam Mali⁸, Anuja Sharad Turkane⁹

DOI: <https://www.doi.org/10.33545/2664844X.2026.v8.i9i.1938>

ABSTRACT

Dry root rot of pigeonpea [*Cajanus cajan* (L.) Millsp.], caused by the soil-borne fungus *Macrophomina phaseolina*, is an increasingly important constraint to pigeonpea production under warm, moisture-deficit conditions. Reliable in vitro cultivation of the pathogen on a nutritionally suitable medium is a prerequisite for its physiological and pathological characterisation, yet the fungus is known to respond differentially to the nutrient composition of the growth substrate. The present study evaluated seven culture media, viz., Potato Dextrose Agar, Richard's Agar, Nutrient Agar, Malt Extract Agar, Yeast Extract Agar, Czapek's Dox Agar and Rose Bengal Agar, for their effect on the mean colony diameter (MCD), colony characteristics and sporulation of *M. phaseolina* under in vitro conditions. A five millimetre disc from a seven-day-old culture was placed at the centre of each medium and observations were recorded after one week of incubation. Mean colony diameter (MCD) was considered the principal quantitative measure of mycelial growth, while sporulation was assessed using a four-grade scale. Colony characteristics were recorded as supporting cultural observations. Significant variation in MCD was observed among the culture media. PDA supported the maximum radial growth, recording an MCD of 90.00 mm, followed by Malt Extract Agar (87.20 mm), Czapek's Dox Agar (78.20 mm), Richard's Agar (75.50 mm), Rose Bengal Agar (75.13 mm), Yeast Extract Agar (54.80 mm) and Nutrient Agar (45.46 mm). PDA also exhibited excellent sporulation (+++++) along with Richard's Agar, whereas Nutrient Agar showed good sporulation (+++). Malt Extract Agar, Yeast Extract Agar and Czapek's Dox Agar exhibited fair sporulation (++) , while Rose Bengal Agar showed scanty sporulation (+). Considerable variation in colony colour, texture, aerial mycelium, margin and comparable growth pattern was recorded among the media. Overall, PDA was the most suitable medium for supporting both rapid radial growth and prolific sporulation of *M. phaseolina* under the conditions of the present investigation.

Keywords: *Macrophomina phaseolina*, Pigeonpea, Dry root rot, Culture media, Colony diameter, Sporulation, M.P.K.V, Rahuri

1. Introduction

Pigeonpea (*Cajanus cajan* [L.] Millsp.) is a diploid ($2n = 16$) grain legume of the Fabaceae family and is recognized for its perennial growth habit, short-day flowering response and robust performance under marginal, rainfed conditions. Originally domesticated in the Indian subcontinent, the crop subsequently migrated to Eastern and Southern Africa and Central America which together with South Asia constitute the three principal production zones worldwide (Nene *et al.*, 1990) [1].

India remains the world's leading producer of pigeonpea, with a national production of approximately 4.34 million tonnes from an area of 5.05 million hectares, corresponding to an average productivity of about 859 kg/ha (DES *et al.*, 2022) [2]. Despite this preminent position, average national productivity continues to lag behind the crop's genetic potential, a shortfall commonly attributed to cultivation under predominantly rainfed and marginal conditions and to exposure to an array of biotic constraints that limit realisable yield (Ghritlahre *et al.*, 2024) [3].

Diseases constitute a particularly important biotic constraint to stable pigeon pea production. The crop is vulnerable to a wide spectrum of diseases caused by fungal, bacterial, and viral pathogens, including phytophthora blight, bacterial leaf spot, sterility mosaic disease, *Alternaria blight*, and *Fusarium wilt* (Nene *et al.*, 1990) [1]. Among these, dry root rot associated with *Macrophomina phaseolina* (Tassi) Goid has emerged as a significant threat, particularly under conditions of elevated temperature and moisture stress. The pathogen is a soil-borne fungus that can remain viable and persist in soil for prolonged periods by producing microsclerotia and pycnidia, which function as significant sources of inoculum (Short *et al.*, 1980;

Corresponding Author:

Aditi Rajesh Joshi
 Department of Plant Pathology and Microbiology, Post Graduate Institute, Mahatma Phule Krishi Vidyapeeth, Rahuri, Maharashtra, India

Gupta *et al.*, 2012) [5, 4]. Infection is initiated through the roots, where the pathogen colonizes cortical and vascular tissues, impairing the uptake and translocation of water and mineral nutrients, ultimately resulting in wilting, premature drying, and significant yield reduction (Kaur *et al.*, 2012) [6].

Macrophomina phaseolina is distinguished 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 consists compact masses of hardened mycelium appears 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) [7]. The pathogen infects a broad host range comprising nearly 500 species belonging to 75 plant families, and high temperature and dry climatic conditions favour disease development (Iqbal & Mukhtar, 2014; Basandrai *et al.*, 2021) [9, 8].

The successful isolation, cultivation, and characterization of *M. phaseolina* under laboratory conditions is fundamental to understanding its biology, nutritional requirements and pathogenic variability. Culture media serve as the primary substrate for fungal growth *in vitro*, providing essential nutrients consisting carbon sources, nitrogen sources, vitamins and minerals that influence mycelial development, sporulation and sclerotial formation. The nutritional composition of culture media can significantly affect growth rate, colony morphology and reproductive structures of fungal pathogens and different media may support varying degrees of growth depending on the specific physiological requirements of the organism (Dhingra *et al.*, 1995) [10].

The present study was carried out to evaluate the effect of various culture media on the growth, sporulation along with colony characteristics of *M. phaseolina* associated with dry root rot of pigeonpea.

2. Materials and methods

The investigation was undertaken under the facilities of the Department of Plant Pathology and Microbiology, Mahatma Phule Krishi Vidyapeeth, Rahuri.

Isolation and identification of pathogen

The pathogen was isolated from infected root tissues obtained from diseased pigeonpea plants collected from field. The recovered isolate was identified through detailed examination of its cultural and morphological attributes, particularly colony characteristics and spore morphology. Microscopic characterization was performed by preparing a temporary mount of the sporulating culture in lactophenol cotton blue stain. The preparation was examined at 400× magnification using a research microscope to record the diagnostic morphological features of the isolate. The pathogenicity of the isolated organism was subsequently established by employing the sick-soil technique.

Preparation of culture media

Seven culture media were evaluated for their effect on the cultural characteristics of *M. phaseolina* viz., Potato Dextrose Agar, Richard's Agar, Nutrient Agar, Malt Extract Agar, Yeast Extract Agar, Czapek's Dox Agar and Rose Bengal Agar. Composition of the each media is as follows :

1. Potato Dextrose Agar (1 lit)

Potato	: 200g
Dextrose :	: 20.00g
Agar-agar:	: 20.00g
Distilled water	: 1000.00 ml

2. Richard's Agar (1 lit.)

Sucrose (C₁₂H₂₂O₁₁)	: 20.00 g
Potassium nitrate (KNO ₃)	: 10.00 g
Potassium dihydrogen phosphate (KH ₂ PO ₄)	: 5.00 g
Magnesium sulphate (MgSO ₄ ·7H ₂ O)	: 2.50 g
Ferric chloride (FeCl ₃ ·6H ₂ O)	: 0.02 g
Agar-agar	: 20.00 g
Distilled water	: 1000.00 ml

3. Nutrient Agar (1 lit.)

Peptone	: 10.00 g
Beef extract	: 3.00 g
Agar-agar	: 20.00 g
Distilled water	: 1000.00 ml

4. Malt Extract Agar (1 lit.)

Malt extract	: 20.00 g
Agar-agar	: 20.00 g
Distilled water	: 1000.00 ml

5. Yeast Extract Agar (1 lit.)

Yeast extract	: 3.00 g
Peptone	: 5.00 g
Glucose	: 10.00 g
Agar-agar	: 20.00 g
Distilled water	: 1000.00ml

6. Czapek's Dox Agar (1 lit.)

Sucrose	: 30.00 g
Sodium nitrate (NaNO ₃)	: 2.00 g
Dipotassium hydrogen phosphate (K ₂ HPO ₄)	: 1.00 g
Potassium chloride (KCl)	: 0.50 g
Magnesium sulphate (MgSO ₄ ·7H ₂ O)	: 0.50 g
Ferrous sulphate (FeSO ₄ ·7H ₂ O)	: 0.01 g
Agar-agar	: 15.00 g
Distilled water	: 1000.00ml

7. Rose Bengal Agar (1 lit.)

Peptone	: 5.00 g
Glucose	: 10.00 g
Potassium dihydrogen phosphate (KH ₂ PO ₄)	: 1.00 g
Magnesium sulphate (MgSO ₄ ·7H ₂ O)	: 0.50 g
Rose Bengal	: 0.05 g
Agar-agar	: 15.00 g
Distilled water	: 1000.00 ml

3. Results and discussion

3.1. Effect of different culture media on mean colony diameter and sporulation of

Macrophomina phaseolina

Significant variation in mean colony diameter of *M. phaseolina* was observed among the seven-culture media evaluated. The mean colony diameter ranged from 45.46 to 90.00 mm. PDA recorded the highest radial growth, recording a mean colony diameter of 90.00 mm and was significantly superior to the remaining media under the conditions of the experiment. Malt Extract Agar ranked second with a mean colony diameter of 87.20 mm. It was followed by Czapek's Dox Agar (78.20 mm), Richard's Agar (75.50 mm) and Rose Bengal Agar (75.13 mm). Yeast Extract Agar recorded a considerably lower mean colony diameter of 54.80 mm, whereas the minimum radial growth was recorded on Nutrient Agar (45.46 mm). The observed values and treatment ranking are depicted in Table 1.

The difference between PDA and Malt Extract Agar was 2.80 mm, which exceeded the reported C.D. of 0.96 mm at 1 per cent probability, indicating significantly greater growth on PDA. Thus, PDA provided the most favourable conditions for radial development of the pathogen among the media evaluated.

The superior performance of PDA in the present investigation is in agreement with several earlier reports. Sahi *et al.* (1992) [11] reported maximum growth on PDA, while Jha *et al.* (2000) [12] recorded the highest growth and sclerotial formation on PDA followed by Richard's medium. Suriachandraselven and Seetharaman (2003) [13] also identified PDA as the best medium among the media evaluated for growth and sclerotial formation. The repeated identification of PDA as a favourable medium suggests that it provides a suitable nutritional environment for vigorous vegetative development of *M. phaseolina*.

sporulation of *Macrophomina phaseolina*

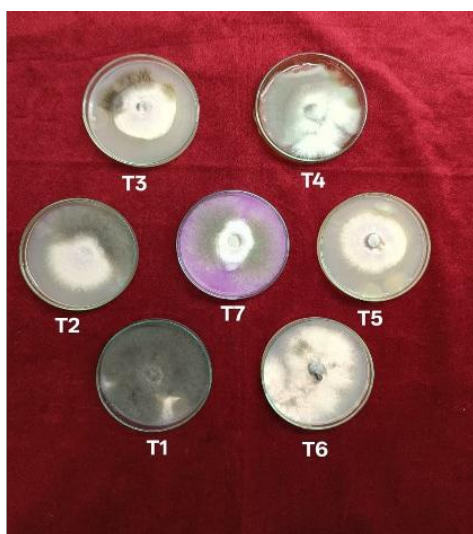


Plate 1. Effect of different culture media on mean colony diameter and

*MCD: Mean colony diameter. Sporulation grade: Scanty (+), Fair (++), Good (+++), Excellent (++++), based on number of conidia per microscopic field (400×).

Table 1. Effect of different culture media on mean colony diameter and

Sr. No.	Culture Medium	MCD (mm)*	Sporulation (Grade)
1	Potato Dextrose Agar	90.00	++++
2	Richard's Agar	75.50	++++
3	Nutrient Agar	45.46	+++
4	Malt Extract Agar	87.20	++
5	Yeast Extract Agar	54.80	++
6	Czapek's Dox Agar	78.20	++
7	Rose Bengal Agar	75.13	+
	S.Em.(±)	0.32	
	C.D. @ 1%	0.96	

sporulation of *Macrophomina phaseolina*

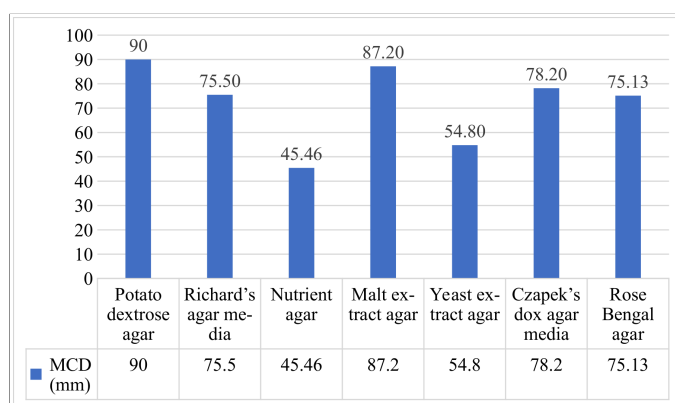


Figure 1. Effect of different culture media on mean colony diameter and

sporulation of *Macrophomina phaseolina*

3.2. Colony characteristics of *Macrophomina phaseolina* on different culture media

Distinct differences in colony morphology were observed among the culture media. PDA produced dark grey to black, dense and velvety to compact colonies with profuse superficial mycelium and a well defined margin. Malt Extract Agar supported white to off-white, highly cottony colonies with profuse aerial mycelium, distinct radial strands and an irregular margin. Czapek's Dox Agar produced white to creamy-white colonies with dark grey to black central pigmentation, abundant aerial mycelium and slight concentric zonation.

On Richard's Agar, colonies were white at the centre and progressively grey to dark grey towards the periphery, with moderately dense cottony to woolly aerial mycelium. Rose Bengal Agar produced white-centred colonies with grey to olivaceous-grey pigmentation, concentric zonation and comparatively dense central aerial mycelium. Yeast Extract Agar developed predominantly white to cream colonies with grey central pigmentation and moderate aerial mycelium, whereas Nutrient Agar produced predominantly white colonies with restricted radial development and grey to dark-grey pigmentation towards the periphery.

The variation in colony colour, texture, aerial mycelium and growth pattern observed in the present study is consistent with earlier reports of cultural variability in *M. phaseolina*. Bairwa *et al.* (2023) documented differences in colony characteristics among *M. phaseolina* isolates, while a study of 60 groundnut isolates reported appressed, fluffy and velvety growth patterns and colony colours ranging from light grey to grey and black.

Media

4. Conclusion

The present study demonstrates that the growth and sporulation of *Macrophomina phaseolina*, the causal agent of dry root rot of pigeon-pea, are significantly influenced by the composition of the culture medium. Among the seven media evaluated, Potato Dextrose Agar supported the maximum mean colony diameter (90.00 mm) along with excellent sporulation, and is therefore identified as the most suitable medium for the vegetative growth and sporulation of this pathogen under *in vitro* conditions, followed by Malt Extract Agar (87.20 mm). These findings confirm Potato Dextrose Agar as an effective standard medium for the laboratory isolation, maintenance and cultural characterisation of *M. phaseolina*.

Acknowledgements

I sincerely acknowledge the support extended by the Post Graduate Institute, Mahatma Phule Krishi Vidyapeeth, Rahuri for providing the necessary facilities and institutional assistance required for carrying out the present investigation. I express my heartfelt gratitude to my Advisor, Dr. S. V. Kolase, for his constant guidance, valuable suggestions, encouragement and careful supervision throughout the course of this research work. I am equally thankful to the members of my Advisory Committee and the faculty and staff of the Department of Plant Pathology and Microbiology, Mahatma Phule Krishi Vidyapeeth, Rahuri for their constructive suggestions, technical guidance and valuable assistance at various stages of the investigation.

Table 2. Colony characteristics of *Macrophomina phaseolina* on different culture

Sr. No.	Culture Medium	Colony Colour	Growth Pattern
1	Potato Dextrose Agar	Predominantly dark, grey to black throughout the colony with a slightly lighter grey centre	Dense, velvety to compact colony with profuse superficial mycelium; radial expansion almost covered the plate; margin entire and well defined
2	Richard's Agar	White at the centre, gradually changing to grey and dark grey towards the periphery	Moderately dense colony with cottony to woolly aerial mycelium; circular colony with a mostly entire margin; fairly uniform radial expansion
3	Nutrient Agar	Predominantly white, with grey to dark grey pigmentation developing unevenly, particularly near the periphery	Fluffy, cottony colony with dense aerial mycelium centrally; uniform radial expansion; margin entire and well defined
4	Malt Extract Agar	White to off-white with light grey central pigmentation and faint grey discoloration towards the periphery	Highly cottony and fluffy with profuse aerial mycelium; rapid radial growth nearly covering the medium; irregular, lobed margin
5	Yeast Extract Agar	Predominantly white to cream with a grey central region; margins lighter than the centre	Compact and cottony colony with moderate aerial mycelium; uniform radial growth with a dense central zone; margin entire, circular and well defined
6	Czapek's Dox Agar	White to creamy white with dark grey to black pigmentation at the centre and light grey patches towards the periphery	Dense, fluffy and cottony colony with abundant aerial mycelium; extensive radial growth with slight concentric zonation; irregular margin
7	Rose Bengal Agar	White central colony surrounded by grey pigmentation, becoming olivaceous grey towards the periphery	Cottony colony with dense aerial mycelium at the centre; moderate to rapid radial growth with concentric zonation; margin entire to slightly diffuse; pigment diffusion into the medium evident

References

- Nene YL, Sheila VK. Pigeon pea: Geography and importance. In: Nene YL, Hall SD, Sheila VK, editors. The Pigeon pea. CAB International, Wallingford, U.K. 1990:1-14.
- DES, MoAF&W. Agricultural Statistics, Fourth Advance Estimates. Directorate of Economics and Statistics, Ministry of Agriculture and Farmers Welfare, Government of India, New Delhi. 2022.
- Ghritlahre SK, Singh A, Singh IP, Singh F, Katiyar PK, Singh NP. Current status and way forward for scaling up productivity of pigeonpea in Madhya Pradesh: A review. *Agricultural Reviews*. 2024;45(2):196-206.
- Gupta O, Patel S, Mishra M. Diversity in isolates of *Rhizoctonia bataticola* causing dry root rot in chickpea from central India. *Jawaharlal Nehru Krishi Vishwa Vidyalyaya Research Journal*. 2012;46(3):376-381.
- Short GE, Wyllie TD, Bristow PR. Survival of *Macrophomina phaseolina* in soil and in residue of soybean. *Phytopathology*. 1980;70:13-17.
- Kaur S, Dhillon GS, Brar SK, Vallad GE, Chand R, Chauhan VB. Emerging phytopathogen *Macrophomina phaseolina*: Biology, economic importance and current diagnostic trends. *Critical Reviews in Microbiology*. 2012;38(2):136-151.
- Lakhran L, Ahir RR, Choudhary M, Choudhary S. Isolation, purification, identification and pathogenicity of *Macrophomina phaseolina* (Tassi) Goid caused dry root rot of chickpea. *Journal of Pharmacognosy and Phytochemistry*. 2018;7(3):3314-3317.
- Basandrai AK, Pandey AK, Somta P, Basandrai D. *Macrophomina phaseolina*-host interface: Insights into an emerging dry root rot pathogen of mungbean and urdbean, and its mitigation strategies. *Plant Pathology*. 2021;70(6):1263-1275.
- Iqbal U, Mukhtar T. Morphological and pathogenic variability among *Macrophomina phaseolina* isolates associated with mung bean (*Vigna radiata* L.) Wilczek from Pakistan. *The Scientific World Journal*. 2014;2014:1-9.
- Dhingra OD, Sinclair JB. Basic plant pathology methods (2nd ed.). Lewis Publishers. 1995.
- Sahi AT, Shakir AS, Bajwa MN, Intezar UI Hassan M. Physiological studies on *M. phaseolina* dry rot of mungbean. *J. Agril. Res. Lahor*. 1992;30(3):409-413.
- Jha AK, Dubey SC. Occurrences of collar rot of okra in the plateau region of Bihar. *Journal of Research, Birsa Agricultural University*. 2000;12(1):67-72.
- Suriachandraselvan M, Seeetharaman K. Effect of culture media on growth and sclerotial production of different isolates of *Macrophomina phaseolina* infecting Sunflower. *J. Mycol. Pl. Patho*. 2003;33(2):226-229.

14. Bairwa PK, Ram D, Choudhary A. Effect of different fungicides against stem and root rot of sesame (*Sesamum indicum* L.) caused by *Macrophomina phaseolina* (Tassi) Goid. Journal of Oilseeds Research. 2022;39(2):122-129.

How to cite this article: Joshi AR, Kolase S, Latake S, Shinde V, Raut S, Sarnobat D, et al. Effect of different culture media on the growth and sporulation of *Macrophomina phaseolina* causing dry root rot of pigeonpea. *International Journal of Agriculture and Food Science*. 2026;8(9):694-698. doi: <https://www.doi.org/10.33545/2664844X.2026.v8.i9i.1938>

Authors

Aditi Rajesh Joshi

Department of Plant Pathology and Microbiology, Post Graduate Institute, Mahatma Phule Krishi Vidyapeeth, Rahuri, Maharashtra, India

SV Kolase

Department of Plant Pathology and Microbiology, Post Graduate Institute, Mahatma Phule Krishi Vidyapeeth, Rahuri, Maharashtra, India

SB Latake

Department of Plant Pathology and Microbiology, Post Graduate Institute, Mahatma Phule Krishi Vidyapeeth, Rahuri, Maharashtra, India

VS Shinde

Sorghum improvement project, Mahatma Phule Krishi Vidyapeeth, Rahuri, Maharashtra, India

SA Raut

College of Agriculture, Malegon, Maharashtra, India

DH Sarnobat

Department of Plant Pathology and Microbiology, Post Graduate Institute, Mahatma Phule Krishi Vidyapeeth, Rahuri, Maharashtra, India

Vaibhavi Vitthalrao Ganjare

Department of Plant Pathology and Microbiology, Post Graduate Institute, Mahatma Phule Krishi Vidyapeeth, Rahuri, Maharashtra, India

Snehal Sudam Mali

Department of Plant Pathology and Microbiology, Post Graduate Institute, Mahatma Phule Krishi Vidyapeeth, Rahuri, Maharashtra, India

Anuja Sharad Turkane

Department of Plant Pathology and Microbiology, Post Graduate Institute, Mahatma Phule Krishi Vidyapeeth, Rahuri, Maharashtra, India