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EFFECT OF DIFFERENT CULTURE MEDIA ON MYCELIAL GROWTH, CULTURAL CHARACTERISTICS AND SPORULATION OF *Colletotrichum lindemuthianum* (Sacc. & Magn.), CAUSING ANTHRACNOSE OF MUNGBEAN (*Vigna radiata* L.)

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ABSTRACT

Mungbean (*Vigna radiata* L.) is an important pulse crop valued for its high nutritional quality, short growth duration and ability to improve soil fertility through biological nitrogen fixation. Anthracnose, caused by *Colletotrichum lindemuthianum* (Sacc. & Magn.), is a major fungal disease that significantly reduces crop yield and seed quality under favourable environmental conditions. Successful isolation, maintenance and mass multiplication of the pathogen are essential for pathological and disease management studies and depend largely on the suitability of the culture medium. Therefore, the present investigation was conducted to evaluate the effect of six different solid culture media on the mycelial growth, cultural characteristics and sporulation of *C. lindemuthianum* under *in vitro* conditions. The pathogen was cultured on Potato Dextrose Agar (PDA), Oat Meal Agar (OMA), Czapek's Dox Agar (CDA), Richards' Agar (RA), Host Leaf Extract Agar (HLEA) and Sabouraud's Agar (SA) using a completely randomized design with three replications. Radial mycelial growth, colony morphology and sporulation were recorded after 10 days of incubation at 25 ± 1°C. Significant differences were observed among the tested media. PDA supported the maximum mycelial growth (90.00 mm) with abundant sporulation, followed by OMA (85.25 mm) and CDA (78.10 mm). Richards' Agar and Host Leaf Extract Agar showed moderate growth, whereas Sabouraud's Agar was the least favourable medium. Variations in colony colour and texture indicated that nutrient composition markedly influenced fungal growth and morphology. The study identified PDA as the most suitable medium for isolation, maintenance and mass multiplication of *C. lindemuthianum*. These findings provide a standardized protocol for future studies on pathogen biology, epidemiology and integrated management of mungbean anthracnose.

***Keywords*:** Anthracnose, *Colletotrichum lindemuthianum*, Culture media, Mungbean, Mycelial growth, Potato Dextrose Agar, Sporulation.

Introduction

Mungbean (*Vigna radiata* L. Wilczek) is one of the most important pulse crops cultivated extensively in tropical and subtropical regions, particularly in India and Southeast Asia. The crop is valued for its short growth duration, high nutritional quality and adaptability to diverse agro-climatic conditions. Mungbean seeds contain approximately 24–26% protein, 60–65% carbohydrates, essential amino acids, vitamins and minerals, making them an inexpensive source of dietary protein. In addition to its nutritional value, mungbean improves soil fertility through symbiotic nitrogen fixation and plays an important role in sustainable cropping systems. Therefore, increasing mungbean productivity is essential for ensuring food and nutritional security.

Among the various constraints affecting mungbean production, fungal diseases are one of the

major causes of yield and quality losses. Anthracnose, caused by *Colletotrichum lindemuthianum* (Sacc. & Magn.), is an economically important disease that infects leaves, stems, petioles and pods, producing reddish-brown necrotic lesions which enlarge and coalesce under favourable environmental conditions. Severe infection results in premature defoliation, pod infection and significant reduction in yield and seed quality. The pathogen survives on infected seeds and crop residues and spreads rapidly under warm and humid conditions, making disease management difficult (Agrios, 2005). The genus *Colletotrichum* comprises one of the most important groups of phytopathogenic fungi, causing anthracnose diseases in numerous agricultural and horticultural crops worldwide. Species of this genus exhibit considerable diversity in morphology, pathogenicity and nutritional requirements, necessitating accurate identification and maintenance under laboratory conditions. Reliable isolation and multiplication of the pathogen are essential for studies on pathogen biology, pathogenicity, host resistance, fungicide evaluation and molecular characterization (Sutton, 1992).

Artificial culture media play a vital role in supporting fungal growth and sporulation by supplying essential nutrients required for metabolism and reproduction. The nutritional composition of the medium influences colony morphology, mycelial growth, pigmentation and conidial production. Since fungal species differ in their nutritional requirements, the selection of an appropriate culture medium is critical for obtaining vigorous growth and uniform inoculum for laboratory studies (Barnett and Hunter, 1998; Alexopoulos *et al*., 1996). Several natural and synthetic media, including Potato Dextrose Agar (PDA), Oat Meal Agar (OMA), Czapek's Dox Agar (CDA), Richards' Agar, Host Leaf Extract Agar and Sabouraud's Agar, have been used for culturing *Colletotrichum* species. Previous studies have reported significant differences in fungal growth and sporulation on different media, with Potato Dextrose Agar generally supporting superior mycelial development and conidial production due to its balanced nutrient composition Koche *et al*. (2011). However, information on the cultural requirements of *C. lindemuthianum* infecting mungbean remains limited.

Therefore, the present investigation was undertaken to evaluate the effect of six commonly used solid culture media on the mycelial growth, cultural characteristics and sporulation of *Colletotrichum lindemuthianum* under *in vitro* conditions. The findings will help identify the most suitable medium for routine isolation, maintenance and mass multiplication of the pathogen and provide a standardized protocol for future studies on the biology and management of mungbean anthracnose.

Materials and Methods

Experimental site

The present investigation was carried out in the Department of Plant Pathology, SKN College of Agriculture, Jobner during 2024–2025 under laboratory conditions. The experiment was conducted to determine the influence of different solid culture media on the mycelial growth, colony characteristics and sporulation of *Colletotrichum lindemuthianum* (Sacc. & Magn.), the causal organism of anthracnose of mungbean. Effect of different media on mycelial growth and sporulation of the pathogen was studied *in vitro* conditions. The growth characters of the fungus were studied on six different solid media *viz*., Potato dextrose agar, Host leaf extract agar, Oat Meal Agar, Czapek`s Dox Agar, Richards' Agar and Sabouraud's Agar media were evaluated to find out the most appropriate medium for the growth of *Colletotrichum lindemuthianum*. All the media were sterilized at 121.6 °C for 20 minutes @15 psi to carry out the study. Twenty ml of each of the medium was poured in to 90 mm diameter Petri dishes. Such plates were inoculated with a disk of five mm diameter of culture and incubated at 25 ± 1 ºC. The experiment was arranged in completely randomized design (CRD) with three replications. The composition of six solid media used during study is given below.

Table 1 : Composition of medium

S. No.	Medium	Constituents	Quantity
1.	Czapeck`s dox agar medium	Agar agar Sucrose Distilled water Dipotassium phosphate Magnesium sulphate Potassium chloride Sodium nitrate Ferus sulphate	15.00 g 30.00 g 1000 ml 1.00 g 0.50 g 0.50 g 2.00 g 0.01g
2.	Oat meal medium	Agar agar Rolled oats Distilled water	15.00 g 30.00 g 1000 ml
3.	Sabouraud's Agar	Dextrose ($C_6H_{12}O_6$) Peptone Agar agar Distilled water	40.00 g 10.00 g 20.00 g 1000 ml
4.	Potato dextrose agar	Peeled potato Agar agar Dextrose Distilled water	200.00 g 20.00 g 20.00 g 1000 ml

5.	Host Leaf Extract Agar	Fresh leaves of green gram Dextrose Agar agar Distilled water	200.0 g 20.0 g 20.0 g 1000 ml
6..	*Richards' Agar*	*Potassium nitrate* *Potassium dihydrogen orthophosphate* *Magnesium sulphate* *Ferric chloride* *Sucrose* *Agar agar* *Distilled water*	*10.0 g* *5.0 g* *2.5 g* *0.02 g* *50.0 g* *20.0 g* *1000 ml*

The degree of sporulation was categorized according to the scale proposed by Pandey and Vishwakarma (1998)**.**

Table 2 : Degree and categories of sporulation

Rate of sporulation	No. of spores/ microscopic field (45X)	Degree of sporulation
Abundant	> 30	++++
Good	21 – 30	+++
Moderate	11 – 20	++
Scanty	< 10	+
Nil	0	-

Observation on mycelial growth

The observations on radial mycelial growth were recorded recorded after 10 days of incubation**.** Colony diameter was measured in two mutually perpendicular directions using a transparent scale, and the average value was expressed in millimetres (mm). Whenever fungal growth completely covered the Petri plate, the maximum colony diameter was recorded as 90 mm.

In addition to radial growth, qualitative observations on colony morphology, including colony colour, colony texture, growth pattern and colony margin, were also recorded.

Assessment of sporulation

The sporulation capacity of the fungus on different culture media was assessed after completion of the incubation period. A 5-mm mycelial disc was excised from the actively growing peripheral region of each colony using a sterile cork borer and transferred into a sterile test tube containing 5 ml of sterile distilled water. The fungal disc was gently macerated using a sterile glass rod to obtain a uniform conidial suspension. One drop of the suspension was placed on a clean glass slide and examined under a compound microscope. Spore counts were recorded from three microscopic fields under 45× objective magnification, and the average number of conidia per microscopic field was calculated.

Experimental design and statistical analysis

The experiment was laid out in a Completely Randomized Design (CRD) comprising six treatments with three replications. Data on radial mycelial growth were subjected to analysis of variance (ANOVA) following standard statistical procedures. Treatment means were compared using the Critical Difference (CD) test at the 5% level of significance (P = 0.05). The Standard Error of Mean (SEm ±) and Coefficient of Variation (CV%) were also calculated to determine the precision and reliability of the experimental observations.

Results

The six culture media evaluated significantly influenced the mycelial growth, colony morphology and sporulation of *Colletotrichum lindemuthianum* under *in vitro* conditions (Table 1 and Plate 1).

Mycelial growth

Significant differences in radial mycelial growth were observed among the tested media after 10 days of incubation. Potato Dextrose Agar (PDA) supported the maximum mycelial growth (90.00 mm), completely covering the Petri plate, followed by Oat Meal Agar (OMA) (85.25 mm) and Czapek's Dox Agar (CDA) (78.10 mm). Richards' Agar (62.18 mm) and Host Leaf Extract Agar (52.24 mm) supported moderate growth, whereas the minimum colony diameter (45.18 mm) was recorded on Sabouraud's Agar (SA).

Colony morphology

Distinct variations in colony morphology were observed on different culture media (Table 1). Colonies on PDA were dense, fluffy, cottony and greyish-white with regular margins. OMA produced white to light-grey, compact and cottony colonies, whereas colonies on CDA were pale grey with smooth and appressed mycelium. Richards' Agar and Host Leaf Extract Agar produced compact white to creamy-white colonies with comparatively slower growth. On Sabouraud's Agar, colonies were sparse with irregular margins and dense aerial mycelium confined mainly to the centre.

Sporulation

The degree of sporulation varied considerably among the tested media. PDA and OMA supported abundant sporulation (++++) followed by CDA, which exhibited good sporulation (+++). Richards' Agar and Host Leaf Extract Agar showed moderate sporulation (++), while Sabouraud's Agar recorded scanty

sporulation (+). Overall, PDA proved to be the most suitable medium for both mycelial growth and conidial production of *C. lindemuthianum* under laboratory conditions.

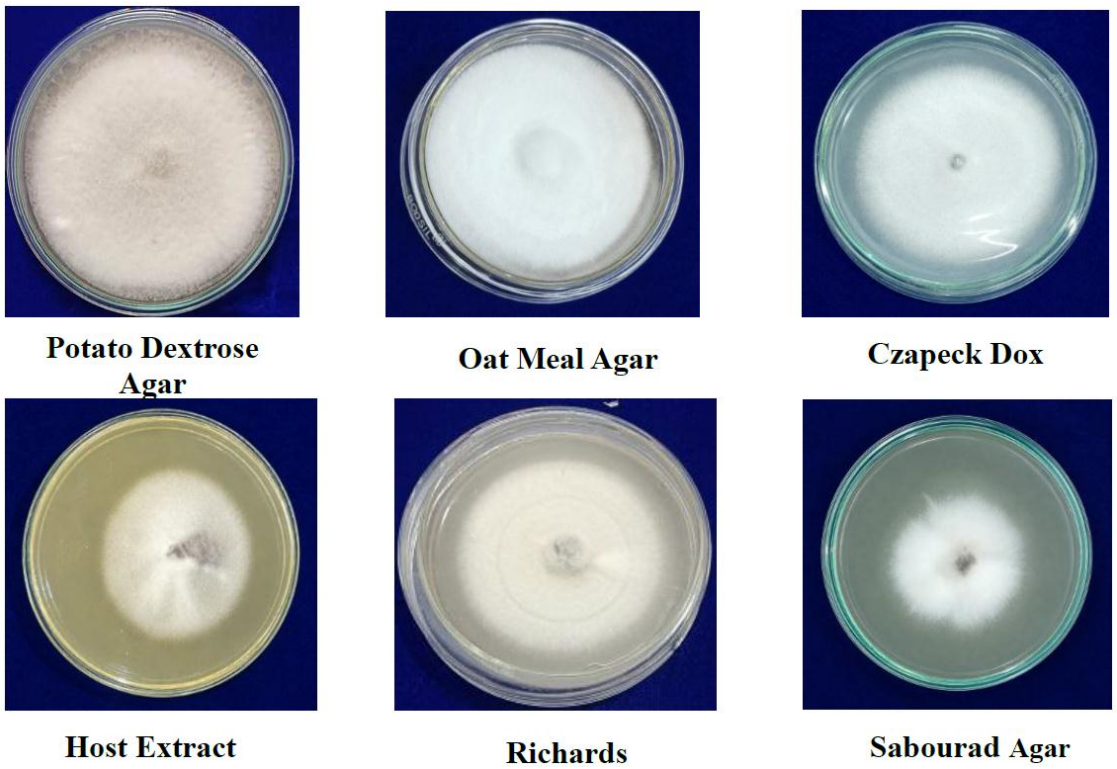

Plate 1: Effect of different solid media on growth and sporulation of *C. lindemuthianum*

Discussion

The present study demonstrated that the composition of culture media significantly influenced the mycelial growth, colony morphology and sporulation of *Colletotrichum lindemuthianum*. Among the six media evaluated, Potato Dextrose Agar (PDA) supported the maximum mycelial growth and abundant sporulation, followed by Oat Meal Agar (OMA), whereas Sabouraud's Agar was the least suitable. These findings indicate that nutrient composition plays a crucial role in the vegetative growth and reproductive development of the pathogen. The superior performance of PDA may be attributed to its balanced nutritional composition, including readily available carbohydrates, vitamins and amino acids supplied by potato infusion and dextrose, which enhance fungal metabolism, hyphal extension and conidial production. Bailey and Jeger (1992) reported that PDA is the most suitable medium for maintaining *Colletotrichum* species because it consistently supports luxuriant mycelial growth, characteristic colony morphology and abundant sporulation. Similar findings were also reported by Than *et al*. (2008) for different *Colletotrichum* species.

Oat Meal Agar also supported excellent fungal growth and sporulation, probably because of its rich content of complex carbohydrates, proteins and vitamins. Similar results were obtained by Sunil and Raja (2019), who reported that PDA followed by Oat Meal Agar was the most suitable medium for the growth and sporulation of *Colletotrichum truncatum* causing anthracnose of mungbean. In contrast, Czapek's Dox Agar supported moderate growth, suggesting that *C. lindemuthianum* responds more favourably to complex natural media than chemically defined media. This observation is consistent with the findings of Cannon *et al*. (2012), who reported that nutritional requirements vary considerably among *Colletotrichum* species. Richards' Agar and Host Leaf Extract Agar supported comparatively lower growth and sporulation than PDA and OMA, while Sabouraud's Agar was the least favourable medium. The poor performance of Sabouraud's Agar may be due to its acidic nature and nutrient composition, which are less suitable for phytopathogenic fungi. Sardhara *et al*. (2016), who observed reduced growth and sporulation of *Colletotrichum* species on nutritionally less favourable media. Marked variations in colony colour, texture, aerial mycelium and colony margins among the tested media indicate that fungal morphology is

strongly influenced by nutrient availability. Comparable variations in cultural characteristics among *Colletotrichum* isolates have also been reported by Hyde *et al*. (2009) and Damm *et al*. (2012).

The pattern of sporulation closely followed mycelial growth, with PDA and OMA producing abundant conidia, CDA showing good sporulation, Richards' Agar and Host Leaf Extract Agar supporting moderate sporulation, and Sabouraud's Agar producing only scanty conidia. Similar relationships between vegetative growth and sporulation have been reported by Manjunath (2009) and Sheelavant *et al*. (2022), who also identified PDA as the most suitable medium for growth and conidial production of different *Colletotrichum* species. Overall, the present findings establish Potato Dextrose Agar as the most suitable medium for the isolation, maintenance and mass multiplication of *C. lindemuthianum*. The abundant mycelial growth and sporulation obtained on PDA make it highly suitable for inoculum production required for pathogenicity studies, resistance screening, fungicide evaluation and other laboratory investigations related to mungbean anthracnose.

Conclusion

The present study demonstrated that culture media significantly influence the mycelial growth, colony characteristics and sporulation of *Colletotrichum lindemuthianum*. Among the six media evaluated, Potato Dextrose Agar (PDA) proved to be the most suitable medium, supporting maximum mycelial growth and abundant sporulation, followed by Oat Meal Agar, whereas Sabouraud's Agar was the least favourable. The observed variation in fungal growth and morphology across different media highlights the importance of nutrient composition in the growth and reproduction of the pathogen. Based on the present findings, PDA can be recommended as the standard medium for routine isolation, purification, maintenance and mass multiplication of *C. lindemuthianum*. The optimized culture conditions established in this study will facilitate inoculum production for pathogenicity testing, resistance screening, fungicide evaluation and other laboratory investigations, thereby supporting future research on the biology and integrated management of mungbean anthracnose.

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