# Assessing the Growth Dynamics and Morphological Traits of *Pyricularia grisea*(Cooke) Sacc. in Response to Media Variation


## ABSTRACT

**Aims:**The objectives of this study were to assess the cultural and physiological requirements for growth and development of *Pyricularia grisea* under *in-vitro*.

**Study design:** Observed data were analyzed by using Completely randomized design with three replications and the critical difference was calculated at 1 % level degree of freedom.

**Methodology:** Present experiments were conducted at laboratory of Plant Pathology in Shaheed Gundadhoor College of Agriculture and Research Station, Jagdalpur. Pure culture of tested pathogen was isolated from finger millet and maintained in potato dextrose agar (PDA) medium for study. Seven different media were prepared and studied for cultural, physiological and morphological characteristics of Pyricularia grisea.

**Results:**The significant differences in colony growth and sporulation were observed over 48, 96, and 144 hours of incubation. Potato Dextrose Agar (PDA) supported the highest colony diameters, ranging from 24.20 mm to 73.20 mm, followed by oatmeal agar (71.40 mm) and finger millet seed extract agar with 2% sucrose (69.80 mm). Czapek dox agar showed the lowest growth (38.40 mm). In terms of sporulation, oat meal agar exhibited the excellent spore production with index of 4 (37 spores per microscopic field), followed by leaf extract agar (33 spores per microscopic field), while Czapek dox agar failed to support sporulation. Colony morphology varied across media: PDA colonies were grayish with concentric rings and rough surfaces; oat meal agar colonies were white with smooth surfaces; finger millet leaf extract agar produced grayish-white colonies with rough surfaces; seed extract agar + 2% sucrose and corn meal agar colonies were black with white edge with smooth surfaces.Seed extract agar colonies were black with white edge with rough surfaces; Czapek dox agar showed poor growth and colonies were black with white edge.

**Conclusion:**These findings highlighted that PDA and oat meal agar are the most suitable media for culturing *P. grisea* for its crucial growth.


***Keywords:****Pyricularia grisea, PDA medium, sporulation and cultural characteristics*

## 1. INTRODUCTION

Millets are category of small-seeded grasses, including species like sorghum, pearl millet, and finger millet (*Eleusine coracana*). Finger millet, also known as Ragi or African millet, is an important crop from the *Poaceae* family, believed to have originated in Ethiopia. It is an allopolyploid with a chromosome count of 2n=4x=36, resulting from hybridization between *Eleusine indica* (AA) and *Eleusine floccifolia* (BB) (Bisht & Singh, 2009).Finger millet, or "poor man's crop," is grown in harsh conditions in Africa and South Asia, ranking third after

sorghum and pearl millet. *Eleusine* is from the Greek city Eleusis, *coracana* from "Kurukkan," and "Ragi" comes from the Sanskrit *Rajika* meaning "red" (Negi *et al.*, 2016).Finger millet is grown in diverse climates across India, especially in Tamil Nadu, Karnataka, Maharashtra, Andhra Pradesh, Gujarat, Madhya Pradesh, Chhattisgarh, and Uttarakhand (Negi *et al.,* 2017).Finger millet is grown on 5 million hectares worldwide, yielding 4.5 million tonnes (Anonymous, 2021). In India, it spans 1.09 million hectares, with a production of 1.73 million tonnes. In Chhattisgarh, it is cultivated on 5.49 thousand hectares, yielding1.39 thousand tonnes (Anonymous, 2021).Finger millet is a resilient, nutrient-dense crop grown for food, fodder and medicine (Mirza & Marla, 2019). Its tolerance to harsh conditions makes it vital for risk management and health foods (Taylor & Emmambux, 2008).It is rich in protein, fiber and minerals like calcium, iron, and zinc. It benefits pregnant women, children, the sick, and diabetics and is essential for small-scale farmers in semi-arid Africa and Asia (Pandey & Kumar, 2005).

Finger millet is vulnerable to diseases like blast, sheath blight and smut, with blast, caused by *Pyricularia grisea*(Cooke) Sacc.,being the most damaging. In India, blast can cause yield losses of 28-36%, and up to 80-90%in affected areas (Nagaraja *et al.*, 2007).*Magnaporthe grisea* (*Pyricularia grisea*) is a destructive fungus affecting finger millet, causing neck and panicle blast. It also infects over 50 grass species, including wheat and rice. The disease causes spindle-shaped lesions with yellow edges, turning white and disintegrating (Thakur, 2023).

This study aims to assess the growth, sporulation, and morphology of *Pyricularia grisea* on various media, and explore the effects of temperature and pH on its growth. The goal is to better understand environmental factors influencing the pathogen, aiding in improved management strategies for finger millet crops.

## 2. MATERIAL AND METHODS

The *in vitro* experiments were conducted at the Plant Pathology laboratory in S.G. CARS, Jagdalpur, Bastar (Chhattisgarh).

### 2.1 The growth, sporulationand morphology of *Pyricularia grisea* on different media.

Growth, sporulation and cultural characteristics of *Pyricularia grisea* were investigated on seven different culture media: Potato Dextrose Agar (PDA), Oatmeal Agar (OMA), Corn Meal Agar (CMA), Czapek's Dox Agar (CDA), Finger Millet Leaf Extract Agar (30%), Finger Millet Seed Extract Agar (10%), and Finger Millet Seed Extract Agar (10%) + 2% sucrose agar. To assess radial growth rate, 7-day-old cultures of *Pyricularia grisea* were inoculated at the center of90 mm Petri plates using 5 mm mycelial discs taken from the

margins of colonies grown on the respective media. The plates were incubated at ambient temperature (25°C), and radial growth was measured at 48,96and144hours post-incubation. Colony characteristics, such as growth rate, topography, and colony color, were visually recorded, along with the total number of conidia per microscopic field. Sporulation was scored on a scale of 0-4, following the descriptions provided by Meena (2005). Morphological features, including conidial size and shape, were analyzed and measured using Motic Image Plus software (version 2.0).

**List 1:Sporulation index of *Pyriculariagrisea***

| Sporulation | No. of spores/microscopic field | Index |
|---|---|---|
| Excellent | >30 | 4 |
| Good | 21-30 | 3 |
| Fair | 10-20 | 2 |
| Poor | <10 | 1 |
| Nil | 0 | 0 |

## 3. RESULTSANDDISCUSSION

**Table 1. Growth of *Pyricularia grisea* in different media.**

### 3.1 Impact of various culture media on the radial growth and sporulation of *Pyricularia grisea*

The cultural characteristics of *Pyricularia grisea* isolated from finger millet were examined on seven different culture media, with results summarized in Table 1,and Fig. 1. Significant differences in average colony diameter (mm) were observed at 48, 96, and 144 hours of incubation, respectively. Among the culture media tested, Potato dextrose agar supported the largest variation in colony diameters, ranging from 24.20mmto 73.20 mm. This was followed by oatmeal agar (71.40 mm), finger millet seed extract agar with 2% sucrose (69.80 mm), finger millet leaf extract agar (51.00 mm), and corn meal agar (46.60 mm). The smallest colony diameters were recorded on Czapek dox agar (38.40 mm) and finger millet seed extract agar (40.40 mm).

| S.No. | Media | Colony diameter (mm) | | | |
|---|---|---|---|---|---|
| | | 48 h | 96 h | 144 h | Mean |
| 1 | Potato dextrose agar | 24.20 | 49.20 | 73.20 | 48.86 |
| 2 | Oat meal agar | 23.64 | 45.20 | 71.40 | 46.74 |
| 3 | Corn meal agar | 6.80 | 27.00 | 46.60 | 26.80 |
| 4 | Czapek dox agar | 8.40 | 23.40 | 38.40 | 23.40 |
| 5 | Leaf extract agar | 12.40 | 38.00 | 51.00 | 33.80 |
| 6 | Seed extract agar | 10.80 | 25.60 | 40.40 | 25.60 |
| 7 | Seed extract agar +2% sucrose | 15.20 | 40.40 | 69.80 | 41.80 |
| | **C.D. at 1%** | **0.67** | **1.15** | **0.82** | |
| | **SE(m)±** | **0.23** | **0.40** | **0.28** | |
| | **C.V.** | **3.57** | **2.49** | **1.13** | |

The effectiveness of different culture media for the sporulation of *Pyricularia grisea* is shown in Table 2 and Plate 2 Fig. 2 Potato dextrose agar supported good sporulation, with 23 spores per microscopic field, an index of 3, pyriform-shaped conidia, and conidia measuring 9.32 µm by 2.86 µm. Oatmeal agar exhibited excellent sporulation, producing 37 spores per microscopic field, an index of 4, and conidia measuring 10.18 µm by 3.92 µm. Corn meal agar also supported good sporulation, with 25 spores per microscopic field, an index of 3, and conidia measuring 9.22 µm by 2.62 µm. In contrast, Czapek dox agar did not support sporulation, with no measurable conidia and a sporulation index of 0. Finger millet leaf extract agar showed excellent sporulation, with 33 spores per microscopic field, an index of 4, and conidia measuring 10.11 µm by 3.82 µm. Finger millet seed extract agar produced moderate sporulation, with 12 spores per microscopic field, an index of 3, and conidia measuring 8.76 µm by 2.29 µm. Similarly, seed extract agar with 2% sucrose supported good sporulation, with 14 spores per microscopic field, an index of 3, and conidia measuring 9.83 µm by 2.68 µm. Similar results were reported by Kumar (2019), who found the highest sporulation (27 spores per microscopic field) and spore size of *P. grisea* on oat meal agar, followed by finger millet seed extract agar + 2% sucrose and finger millet seed extract agar. Malviya (2014) noted that among various media, PDA was the most suitable for the growth and sporulation of *P. grisea*.

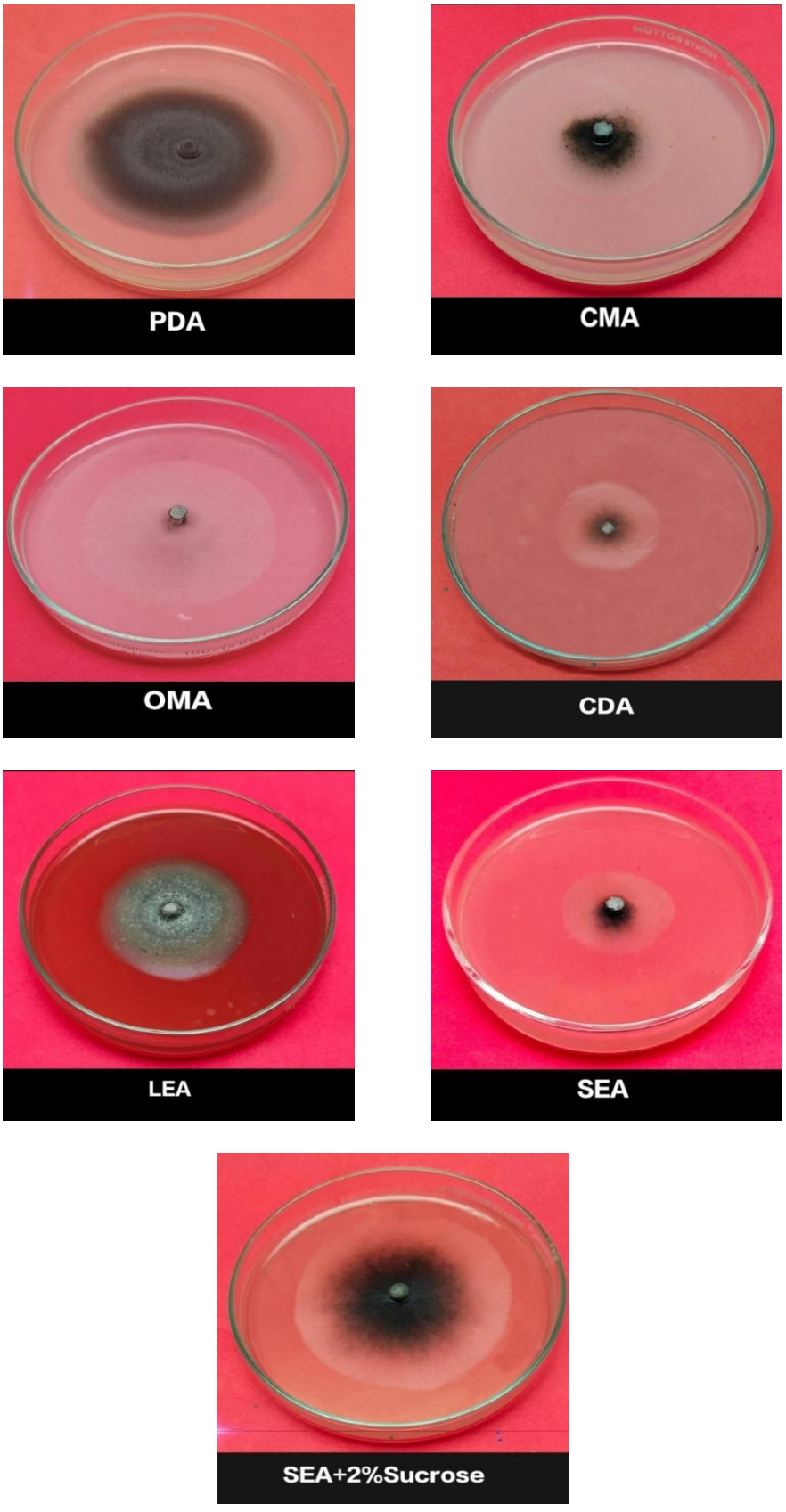


**Fig. 1. Variations in colony morphology of *Pyricularia grisea* in different media**

**Table 2. Spore characteristics of *Pyricularia grisea* on different culture media.**

| S.No. | Media | Shape | Sporulation type | No. of spores/microscopic field | Index | Septa | Conidia size (µm) (40X) | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | Length | Width |
| **1** | **Potato dextrose agar** | Pyriform | Good | 23 | 3 | 2 | 9.32 | 2.86 |
| **2** | **Oat meal agar** | Pyriform | Excellent | 37 | 4 | 2 | 10.18 | 3.92 |
| **3** | **Corn meal agar** | Pyriform | Good | 25 | 3 | 2 | 9.22 | 2.62 |
| **4** | **Czapek dox agar** | Pyriform | Nil | 0 | 0 | 0 | 0 | 0 |
| **5** | **Leaf extract agar** | Pyriform | Excellent | 33 | 4 | 2 | 10.11 | 3.82 |
| **6** | **Seed extract agar** | Pyriform | Good | 12 | 3 | 2 | 8.76 | 2.29 |
| **7** | **Seed extract agar +2% sucrose** | Pyriform | Good | 14 | 3 | 2 | 9.83 | 2.68 |

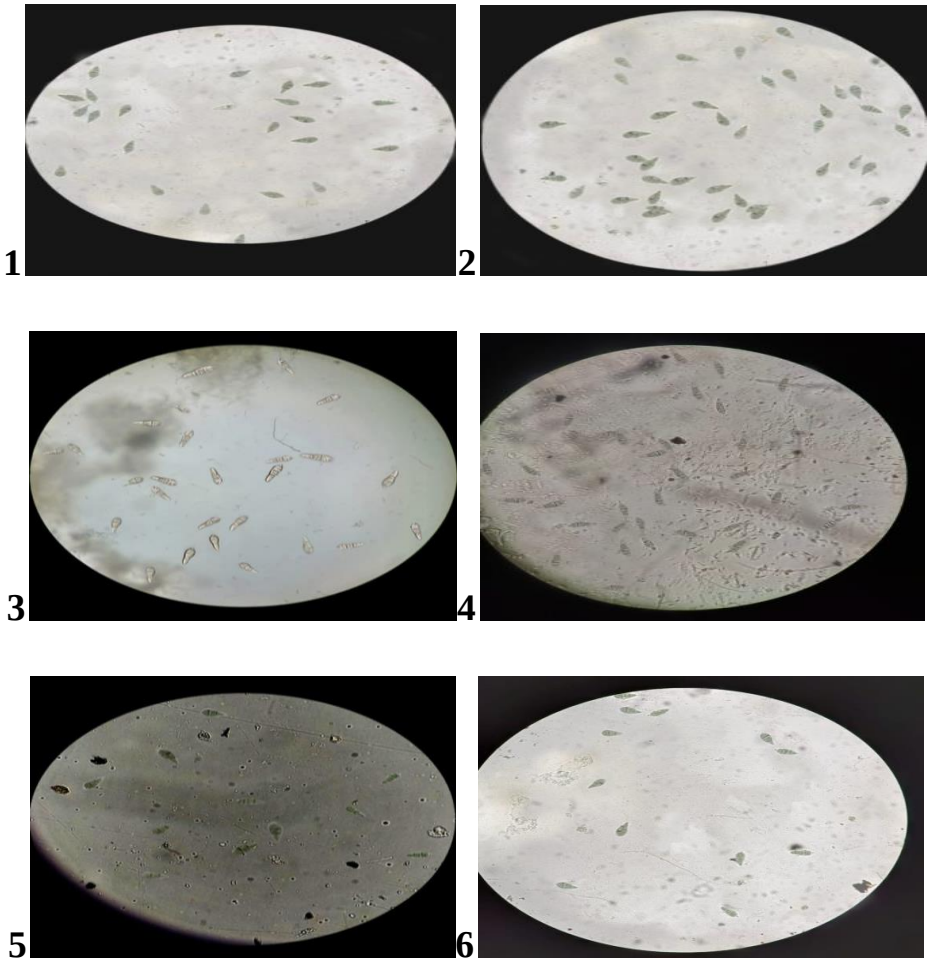


**Fig. 2.Spore characteristics of *Pyricularia grisea* on different culture media 1 PDA,2 OMA,3 CMA,4 LEA, 5 SEA, 6 SEA+2%Sucrose.**

## 3.2 Culture characteristics of *Pyricularia grisea* on different culture media.

The cultural characteristics of *Pyricularia grisea* were studied on different media, Table 3, and Fig. 1 showing significant variation in growth, colony morphology, and shape. potato dextrose agar and oat meal agar supported the good growth, while czapek dox agar showed poor growth. Most colonies appeared black with white edges, except for gray colonies on potato dextrose agar, greyish white on leaf extract agar and white colonies on oat meal agar. The colonies were consistently regular in shape across all media. Concentric rings were observed only on potato dextrose agar and leaf extract agar. Surface structures were mostly smooth, with rough surfaces on potato dextrose agar, leaf extract agar and seed extract agar. Pigmentation was absent in all cases. Overall, Potato Dextrose Agar and oat meal agar was the most suitable for studying *P. grisea.*

Vanaraj*et al.* (2013) obtained similar results when they tested five different natural media (oatmeal agar, rice agar, rice polish agar, malt extract agar, and potato dextrose agar) to

isolate *P. oryzae*. They found that the pathogen's mycelial growth was fastest on PDA and malt extract agar. Additionally, the colonies of *P. oryzae* appeared white on oatmeal, rice polish, and malt extract agars, while they were grey on potato dextrose agar. Similarly, Kulmitra *et al.* (2017) investigated the cultural characteristics of seven *P. oryzae* isolates, focusing on colony traits such as growth type and colony color. They reported that on PDA medium, the isolates produced colonies ranging from white to slightly grayish-white, while on oatmeal agar, two isolates formed black colonies, and the remaining isolates developed white colonies.

**Table 3. Cultural characteristics of *Pyricularia grisea* in different culture media**

| S.No. | Media | Growth character | Color | Surface structure / character | Shape | Concentric ring | Pigmentation |
|---|---|---|---|---|---|---|---|
| **1** | Potato dextrose agar | Good | Grey | Rough | Regular | Present | No |
| **2** | Oat meal agar | Good | White | Smooth | Regular | Absent | No |
| **3** | Corn meal agar | Medium | Black with White edge | Smooth | Regular | Absent | No |
| **4** | Czapek dox agar | Poor | Black with White edge | Smooth | Regular | Absent | No |
| **5** | Leaf extract agar | Medium | Greyish White | Rough | Regular | Present | No |
| **6** | Seed extract agar | Medium | Black with White edge | Rough | Regular | Absent | No |
| **7** | Seed extract agar +2% sucrose | Medium | Black with White edge | Smooth | Regular | Absent | No |

## 4. CONCLUSIONS

The study examined the growth, morphological traits, and sporulation of *P. grisea* across seven different media and varying environmental conditions. The fungus showed the best growth on PDA, with a mean growth rate of 48.86 mm, followed closely by Oatmeal agar. Czapek dox agar supported the poorest growth. The addition of 2% sucrose to Seed extract agar significantly enhanced growth. Morphologically, colony appearance and texture varied by medium, with PDA colonies being grey and rough, while Oatmeal agar produced smooth, white colonies. Sporulation was highest on Oatmeal agar and Leaf extract agars, producing the largest conidia. These results indicate that *P. grisea* grows best under specific nutrient conditions, particularly on PDA and Oatmeal agar.

## DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declares that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

## COMPETING INTERESTSA

Authors have declared that no competing interests exist

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## AUTHORS' CONTRIBUTIONS

The authors collaborated on all aspects of this work. Each author contributed to the research and manuscript preparation, and all authors reviewed and approved the final version of the manuscript. Additionally, it's important to mention that the collective input from all authors helped in ensuring the quality and accuracy of the research. This collaborative approach allowed for diverse expertise and perspectives to be incorporated, enhancing the overall depth and reliability of the findings.

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