

Assessing the Growth Dynamics and Morphological Traits of *Pyricularia grisea* in Response to Media Variation

ABSTRACT

The cultural characteristics of *Pyricularia grisea* isolated from finger millet were evaluated across seven different culture media. Significant differences in colony growth and sporulation were observed over 48, 96, and 144 hours of incubation. Potato Dextrose Agar (PDA) supported the largest 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 smallest 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. Pigmentation was absent in all culture media. These findings highlight that PDA and oat meal agar are the most suitable media for culturing *P. grisea* crucial for its growth.

Key words: *Pyricularia grisea*, media, PDA, oat meal agar

INTRODUCTION

Millet is a 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; Chennaveeraiah & Hiremath, 1967). Finger millet, or "poor man's crop," is grown in harsh conditions in Africa and South Asia, ranking third after sorghum and pearl millet (Hilu & de Wet, 1976). *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, yielding 1.39 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 (Shastri, 1989; 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 (Govindu & Shivanandappa, 1967). 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 (Ou, 1985). 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.

MATERIAL AND METHODS

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

The growth, sporulation and 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), Oat meal 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 of 90 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, 96, and 144 hours 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 *Pyricularia grisea*

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

RESULT AND DISCUSSION

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 Plate 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 24.20 mm to 73.20 mm. This was followed by oat meal 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).

The effectiveness of different culture media for the sporulation of *Pyricularia grisea* is shown in Table 2 and Plate 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 oatmeal agar, followed by finger millet seed extract agar + 2% sucrose and finger millet seed extract agar. Awoderuet *et al.* (1991) also reported optimal mycelial growth of *M. oryzae* on PDA. Malviya (2014) noted that among various media, PDA was the most suitable for the growth and sporulation of *P. grisea*.

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

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	

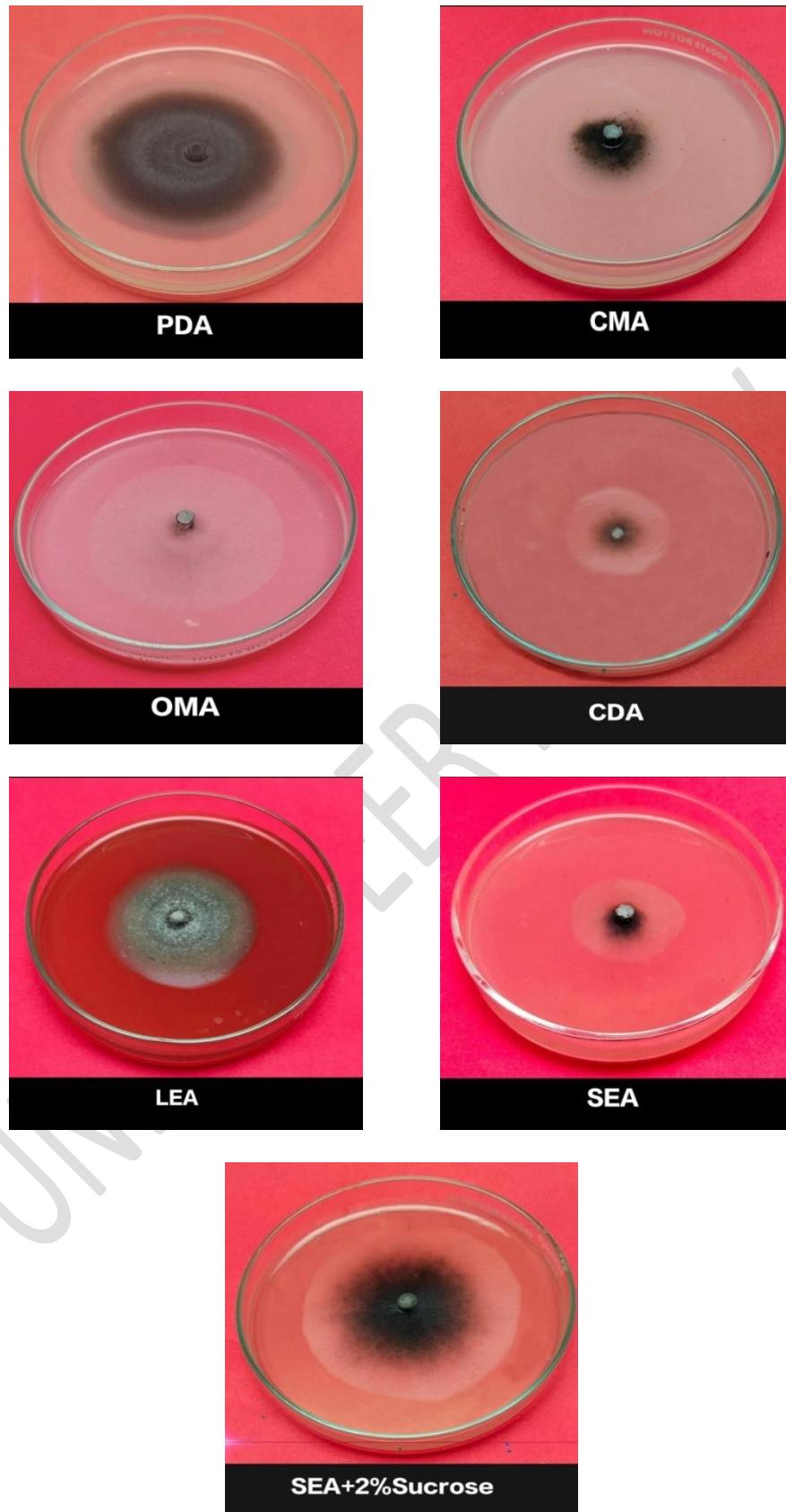


Plate 1. Variations in colony morphology of *Pyricularia grisea* in different media

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	Pyriiform	Good	23	3	2	9.32	2.86
2	Oat meal agar	Pyriiform	Excellent	37	4	2	10.18	3.92
3	Corn meal agar	Pyriiform	Good	25	3	2	9.22	2.62
4	Czapek dox agar	Pyriiform	Nil	0	0	0	0	0
5	Leaf extract agar	Pyriiform	Excellent	33	4	2	10.11	3.82
6	Seed extract agar	Pyriiform	Good	12	3	2	8.76	2.29
7	Seed extract agar +2% sucrose	Pyriiform	Good	14	3	2	9.83	2.68

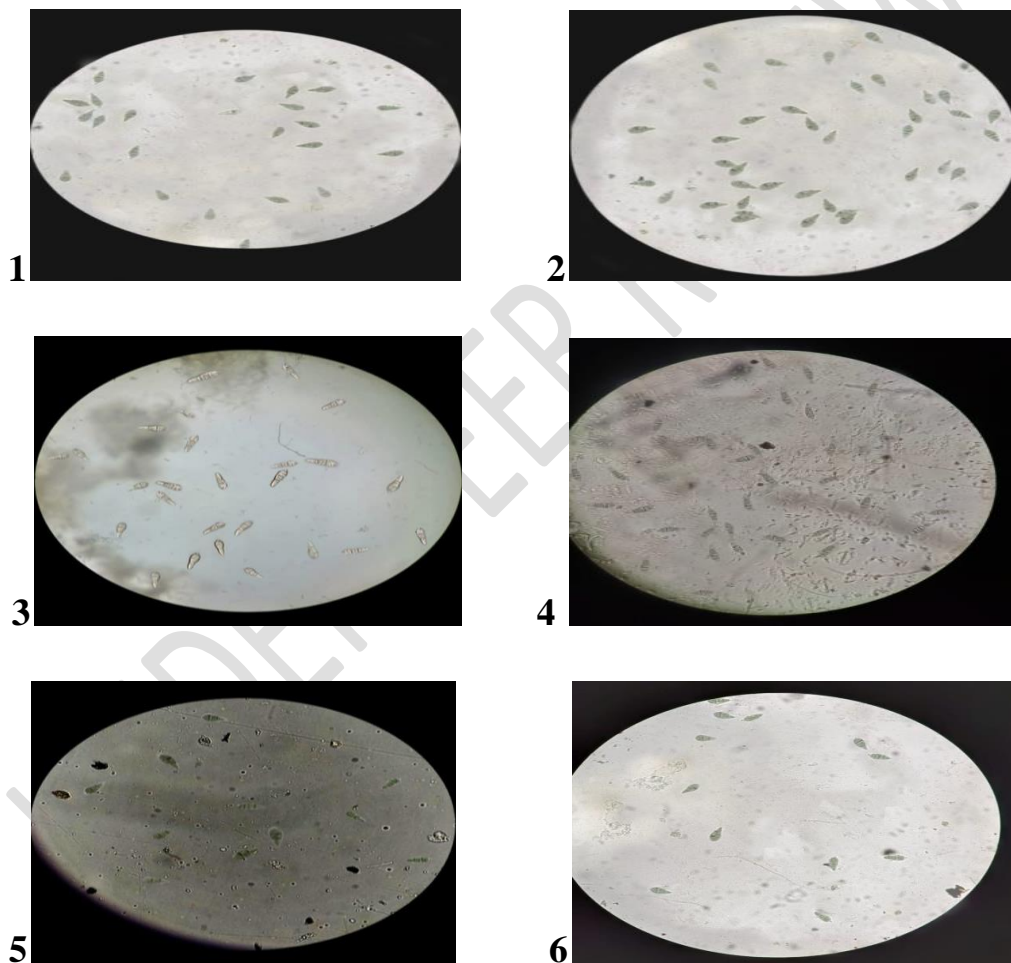


Plate 2. Spore characteristics of *Pyricularia grisea* on different culture media 1 PDA , 2 OMA , 3 CMA , 4 LEA , 5 SEA , 6 SEA+2%Sucrose.

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

The cultural characteristics of *Pyricularia grisea* were studied on different media, Table 3, and Plate 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 were 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 oat meal, 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.N o.	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	Medium	Black with White edge	Rough	Regular	Absent	No

	t agar						
7	Seed extrac t agar +2% sucro se	Medium	Black with White edge	Smooth	Reg ular	Absent	No

CONCLUSION

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 Potato Dextrose Agar (PDA), with a mean growth rate of 48.86 mm, followed closely by Oat meal Agar. Czapek 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 Oat meal Agar produced smooth, white colonies. Sporulation was highest on Oat meal and Leaf Extract Agars, producing the largest conidia. These results indicate that *P. grisea* grows best under specific nutrient conditions, particularly on PDA and Oat meal Agar.

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