

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

2 597 WORDS / 3000-6000 WORDS

ABSTRACT 238 WORDS / 300 WORDS

The cultural characteristics of *Pyricularia grisea* isolated from finger millet were evaluated across seven different culture media. ??OBJETIVE????

METHODOLOGY??????

?RESULTS???

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.

?? CONCLUSIONS????

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

1- 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

Comment [AEFH1]: 1. Original research papers:

Papers that include original empirical data that have not been published anywhere earlier (except as an abstract). Null/negative findings and replication/refutation findings are also welcome. This type of paper normally should not exceed 25 double-spaced pages of text (including references) and should not contain more than 15 figures/tables. We advise a length of 3000-6000 words (including everything).

Comment [AEFH2]: Nomen binomial *Magnaporthe grisea* (T.T. Hebert) M.E. Barr

Sinónimos
Ceratospheeria grisea T.T. Hebert, (1971)
Dactylaria grisea (Cooke) Shirai, (1910)
Dactylaria oryzae (Cavara) Sawada, (1917)
Phragmoportha grisea (T.T. Hebert) M. Monod, (1983)

Pyricularia grisea Sacc., (1880)
 (anamorfo)
Pyricularia grisea (Cooke) Sacc., (1880)
Pyricularia oryzae Cavara, (1891)

Trichothecium griseum Cooke,
Trichothecium griseum Speg., (1882)

Comment [AEFH3]: REFORMULATE THE ABSTRACT: USE THE JOURNAL TEMPLATE.

Comment [AEFH4]: Manuscript structure

The manuscript should be written in English with a simple layout. The text should be prepared in single column format. Bold face, italics, subscripts, superscripts etc. can be used. The text, excluding the abstract, if required, can be divided into numbered sections with brief headings. Starting fr...

Comment [AEFH5]: *Eleusine coracana* (L.) Gaertn.

Nomen binomial
Eleusine coracana Gaertn.

Sinónimos [1]
Cynodon coracanus Raspail
Cynosurus coracanus L.

Comment [AEFH6]: For biological nomenclature, the conventions of the International Code of Botanical Nomenclature, the International Code of Nomenclature of Bacteria, and the International Code of Zoological Nomenclature should be followed.

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

Comment [AEFH7]: INCOMPLETE INFORMATION. ? COUNTRY, ?.

S.G. College of Agriculture and Research Station, Jagdalpur, India.

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*

Comment [AEFH8]: List or Table?????

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*

Comment [AEFH9]: The text, excluding the abstract, if required, can be divided into numbered sections with brief headings. Starting from introduction with section 1. subsections should be numbered (for example 2.1 (then 2.1.1, 2.1.2, 2.2, etc.), up to three levels.

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.

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	

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*.

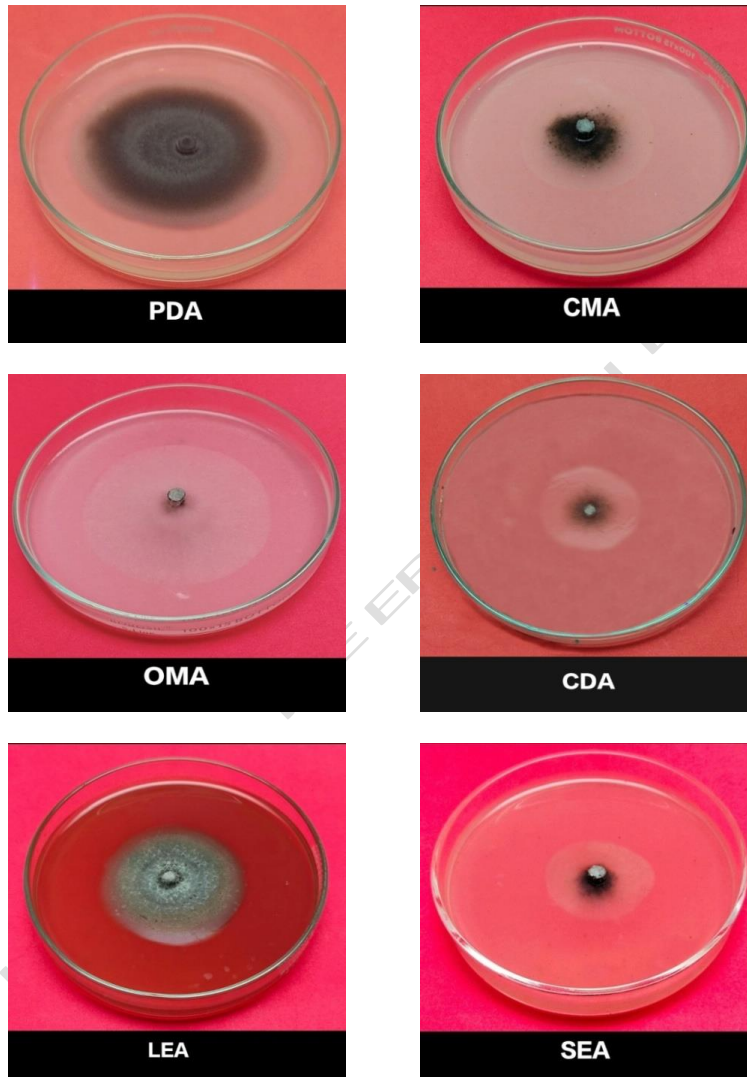




Plate 1. Variations in colony morphology of *Pyricularia grisea* in different media

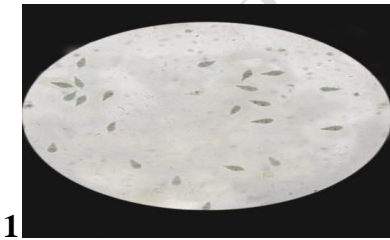
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Table 2. Spore characteristics of *Pyricularia grisea* on different culture media.

S.N o.	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

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PEER REVIEW



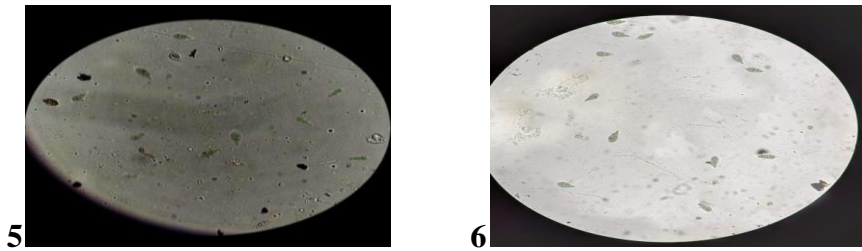


Plate 2. Spore characteristics of *Pyricularia grisea* on different culture media 1 PDA , 2 OMA , 3 CMA , 4 LEA , 5 SEA , 6 SEA+2%Sucrose.

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.No.	Media	Growth character	Color	Surface structure / character	Shape	Concentric ring	Pigmentation
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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

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.

References must be listed at the end of the manuscript and numbered in the order that they appear in the text. wrong in alphabetical order

REFERENCES :

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References must be listed at the end of the manuscript and numbered in the order that they appear in the text. Every reference referred in the text must also present in the reference list and vice versa. In the text, citations should be indicated as (Author name, year).

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Malviya R. (2014) tested four media namely Potato dextrose agar, Czapek's Dox Agar, Rechar Agar medium and Corn meal agar. However, PDA was found to be highly suitable for the growth and sporulation of *P.grisea*.

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Comment [AEFH16]: All references should follow the following style:

Reference to a journal:

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1. Hilly, M., Adams, M. L., & Nelson, S. C. (2002). A study of digit fusion in the mouse embryo. Clinical and Experimental Allergy, 32(4), 489-498.

Note: List the first six authors followed by et al.

Note: Use of a DOI number for the full-text article is encouraged. (if available).

Note: Authors are also encouraged to add other database's unique identifier (like PUBMED ID).

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Reference in list+= 22. 17 (2005-2023); Media= 2014 (10 YEARS OLD).
5 (1967-1989); Media= 1977 (47 YEARS OLD)
MEDIA GERAL = 2005. (19 YEARS OLD).