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

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Authors' contributions

This work was carried out in collaboration among all authors. 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. All authors read and approved the final manuscript.

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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 oat meal 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 yielding worldwide. 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 tones

(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, 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), 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 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 and144 hours postincubation. 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 provided by Meena (2005). descriptions 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	

3. RESULTSANDDISCUSSION

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 24.20 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).

Table 1. Growth of	Pyricularia	grisea in	different	media
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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 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. Oat meal 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.





Pujari et al.; Asian J. Adv. Agric. Res., vol. 24, no. 12, pp. 34-42, 2024; Article no.AJAAR.128588





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

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 (oat meal 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.

S.No.	Media	Shape	Sporulation type	No. of spores/microscopic	Index	Septa Conidia size (µm) (40)		e (µm) (40X)
				field			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

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

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

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

Pujari et al.; Asian J. Adv. Agric. Res., vol. 24, no. 12, pp. 34-42, 2024; Article no.AJAAR.128588



5

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

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.

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 Oat meal agar. Czapek dox agar supported the poorest growth. The addition of 2% sucrose to Seed extract agar significantly growth. Morphologically, colony enhanced 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 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 oat meal 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.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Pujari et al.; Asian J. Adv. Agric. Res., vol. 24, no. 12, pp. 34-42, 2024; Article no.AJAAR.128588

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