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Comparative evaluation of different media and pH for the culturing of *Bipolaris setariae* causing leaf blight in browntop millet in India

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Abstract

Browntop millet was newly included in the millet cropping system in India as one of the small millet. Incidence of leaf blight was observed to be severe in all millet growing regions of India and the causal organism was confirmed as *B. setariae* using molecular tools viz., ITS and *GPDH* genes. As the crop was newly included in the millet system, investigation was needed to carry out about disease causing agents their biology and culture conditions. In this study, an attempt was made to identify the ideal culturing and physiological requirements for culturing *B. setariae*. Results revealed that solid media such as Czapek Dox agar (CDA) and host leaf decoction agar with 2 per cent sucrose are ideal for better growth and abundant sporulation while, pH 6.0 to pH 7.0 were found to be ideal for radial mycelial growth and sporulation. These cultural and physiological conditions can be used for the future studies while working on the pathogen causing browntop millet leaf blight.

Keywords: browntop millet, *B. setariae*, *In vitro*, media, Czapek Dox agar and pH

1. Introduction

Millets have been superior to commercially growing cereal crops since ancient times not in terms of quantity that they produce but in terms of quality that they possess. With their importance and considering on going climate changes, FAO approved and declared 2023 as the International year of millets whereas, India celebrated 2018 as the National year of millets. Small millets are one of the crops that are negligible in production but are highly nutritious and also possess features like drought tolerance, high mineral and vitamin content. Browntop millet [*Brachiaria ramosa* (L.) Stapf] native to India and was recently included into millets system in India as one of the small millet for commercial cultivation. It is different from other small millets by having characters like short growth period, shade tolerant, suppress root knot nematode and because of their sharp and stiff leaf structure it obstructs the entry of rats in to fields. In India, it is majorly cultivated in dry tracts of Andhra Pradesh-Karnataka border areas, Tamil Nadu and Maharashtra (Sujata *et al.*, 2018) ^[1]. Among the various biotic constraints, diseases caused by fungal pathogens are wide spread and destructive. Fungal disease recorded on small millets includes blast, leaf blight/brown spot, smut, downy mildew, leaf spot etc., among all the diseases brown spot or leaf blight disease caused by *Helminthosporium* sp. is gaining importance next only to blast in different parts of India. Misra and Prakash (1972) ^[11] first observed association of *H. setariae* with leaf spot of browntop millet. But there are no systemic studies were carried out on pathogen infecting browntop millet. The Present study concentrated on the culturing aspects of *B. setariae* to find ideal media and pH for better growth and sporulation which is useful for future studies on various aspects of the pathogen.

2. Material and Methods

2.1 Effect of different media

The study was conducted to identify ideal medium and to describe the cultural characteristics viz., colour, type of margin, surface, topography, pigmentation, texture and sporulation of the pathogen and morphological characteristics of hyphal, conidiophore and conidia on different solid media. 5 mm culture discs of the pathogen were inoculated separately on different media

viz., PDA, corn meal agar, malt extract agar, Sabouraud's medium, Richard's agar medium, Czapek Dox agar, rose Bengal agar, oat meal agar, host leaf decoction agar, host leaf decoction and 2 per cent sucrose agar medium, vegetable juice (V₈) agar, water agar medium and were incubated at 27±1 °C for seven days. The cultural characteristics and the colony diameter (mm) on each medium were recorded. The composition of different solid media used during the study is given below (Waller *et al.*, 2001) [3]. All the chemical ingredients excluding agar were dissolved in 500 ml water and agar was melted separately in distilled water (500 mL). Both the solutions were mixed thoroughly and medium was sterilized at 1.1 kg/cm² pressure for 15 min and preserved for further use. 20 ml of each solid medium was poured into 90 mm diameter Petri plates and replicated thrice. Five mm culture disc of pathogen was taken from the periphery of 10 days old colony grown on PDA and placed at the centre of the plate. Such plates were incubated at 27±1 °C for 15 days. The sporulation was graded as follows.

Table 1: Sporulation rate and symbols

Sl. No	Grade	Conidia per (10x) microscopic field	Symbol/ Indication
1	Excellent	>70	++++
2	Good	46-70	+++
3	Moderate/ Limited	16-45	++
4	Poor / Less	1-15	+
5	No sporulation	-	-

For sporulation estimation, a five mm disc of the culture was cut from random places of the plate and suspended in sterilized water (10 ml) and shaken well, so that the spores were dislodged. One drop of this spore suspension was placed on a haemocytometer and the numbers of spores in 4 squares at random, were counted. The number of spores per ml was calculated with a haemocytometer, using the formula: No. of spores/ml (N) = n×16×10³, where, N = Total No. of spores counted/ml, n = Average number of spores in each smallest square of haemocytometer.

2.2 Effect of Hydrogen ion concentration (pH) in liquid and solid medium.

This study was conducted to know the influence of pH on growth and sporulation of pathogen. pH of PDA and PDB was adjusted to *viz.*, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5 and 8.0 with 1N Hydrochloric acid (HCl) and 1N Sodium hydroxide (NaOH) by using digital pH meter. Each treatment was replicated thrice. The pH adjusted media was autoclaved at 121 °C and 15 lbs pressure for 15 min. 15 to 20 mL of PDA was poured into Petri plate in case of solid media and was seeded with 7 mm disc of 15 days old mycelial culture and

mycelial growth was observed periodically and the diameter of the colony was measured after 10 days of incubation. However, the mycelial mat on PDB was harvested and filtered by using previously weighed what man No.1 filter paper and at 60 °C in a hot air oven until the dry weight readings become constant. The difference in weight of mycelia was averaged and analysed statistically (Aneja, 2001) [4].

3. Result and Discussion:

3.1 Influence of different culture media on growth and sporulation

Among the 12 media, the highest significant radial growth was recorded in Czapek Dox agar (90.00 mm) where other media showed variation in growth ranging from 23.55 - 87.22 mm and very little growth in the form of thin mycelial mat was observed in water agar at 6 days after inoculation. The pathogen in Czapek Dox agar had covered full Petri plate within 6 days after inoculation with full pigmentation on reverse side of the plate in 7 days after inoculation while mycelial growth in other media had covered full plate between 7-12 days and full pigmentation varied from 10-18 days. Full cover of mycelial growth and pigmentation was not observed in V8 juice agar and water agar. The colony colour was observed to be bright grey in Czapek Dox agar and malt extract agar where other media showed grey to greyish white (Table 2, Plate 1 and Fig. 1). The colony texture was variable with loose to fluffy in most of the media while the compact texture was observed in PDA and oat meal agar. The colony margin was regular and while in some it was irregular, but curled margin was noticed in corn meal agar. All the solid media exhibited dull luster of the mycelial growth.

Sporulation of *Bipolaris* sp. was observed to be comparably high in host leaf decoction agar + 2 per cent Sucrose and potato dextrose agar (46-70 conidia per microscopic field), but the lowest sporulation was noticed on corn meal agar and vegetable juice agar (10 conidia per microscopic field). No sporulation was recorded in Sabouraud's dextrose agar and rose Bengal agar. Among the 12 media evaluated, Czapek Dox agar (CDA) and host leaf decoction agar with 2 per cent sucrose was found to be ideal for radial mycelial growth and spore production by *Bipolaris* sp. infecting browntop millet. The results are in agreement with Sinijadas *et al.* (2018) [5] who tested different solid media for *B. sorokiniana*, where the growth of the colony in CDA, PDA and OMA was blackish grey coloured with irregular margins and Rangaswami and Pandurangan (1962) [6] observed *B. oryzae* growing best on Czapek Dox media. Similarly, Tanaka (1956) [7] observed CDA along with maltose as a carbon source as best medium to grow *B. oryzae*. Nayak and Hiremath (2019) [8] observed the maximum growth of *B. oryzae* on PDA followed by host leaf dextrose agar.

Table 2: Effect of different media on cultural characteristics of *B. setariae* (BTMHs isolate) infecting browntop millet

Sl. No.	Media	Days to cover full plate*	Days to full pigmentation*	Radial mycelial growth (mm)*	Colour	Colony texture	Surface and topography	Margin	Luster	Sporulation
1	Czapek Dox Agar (CDA)	6	7	90.00	Bright grey	Loose and Fluffy	Uniform and umbonate	Regular	Dull	++
2	Richard's Synthetic Agar (RSA)	7	10	85.99	Greyish white	Fluffy	Uniform and raised	Regular	Dull	+
3	Host leaf decoction Agar (HLA)	7	15	87.22	Greyish white	Loose	Uniform and raised	Regular	Dull	+
4	Host leaf decoction Agar (HLA) + 2 % Sucrose	7	12	83.99	Grey	Loose and fluffy	Uniform and raised	Regular	Dull	+++
5	Oat Meal Agar (OMA)	7	11	80.77	Greyish white	Compact	Uniform and umbonate	Regular	Dull	+
6	Malt Extract Agar (MEA)	8	15	78.55	Bright grey	Loose	Uniform and flat	Regular	Dull	+
7	Corn Meal Agar (CMA)	9	16	63.00	Greyish white	Loose and fluffy	Wavy and raised	Curled	Dull	+
8	Potato Dextrose Agar (PDA)	9	12	51.44	Grey	Compact	Wavy and flat	Irregular	Dull	+++

9	Sabouraud's Dextrose Agar (SDA)	11	17	49.77	Greyish white	Moderately compact	Uniform and raised	Irregular	Dull	-
10	Rose Bengal Agar (RBA)	12	18	46.22	Greyish white	Moderately compact	Wavy and flat	Irregular	Dull	-
11	Vegetable juice (V8) Agar	-	-	23.55	Light grey	Loose	Wavy and flat	Irregular	Dull	+
12	Water Agar (WA)	-	-	Very less growth was observed and it forms as thin layer of grey colour mycelium						
	S.Em±	0.44								
	CD (P 0.01)	1.52								

Note: * Mean of three replications; - : no sporulation; + : 1-15 conidia; ++ : 16-45 conidia; +++: 46-70 conidia per microscopic field

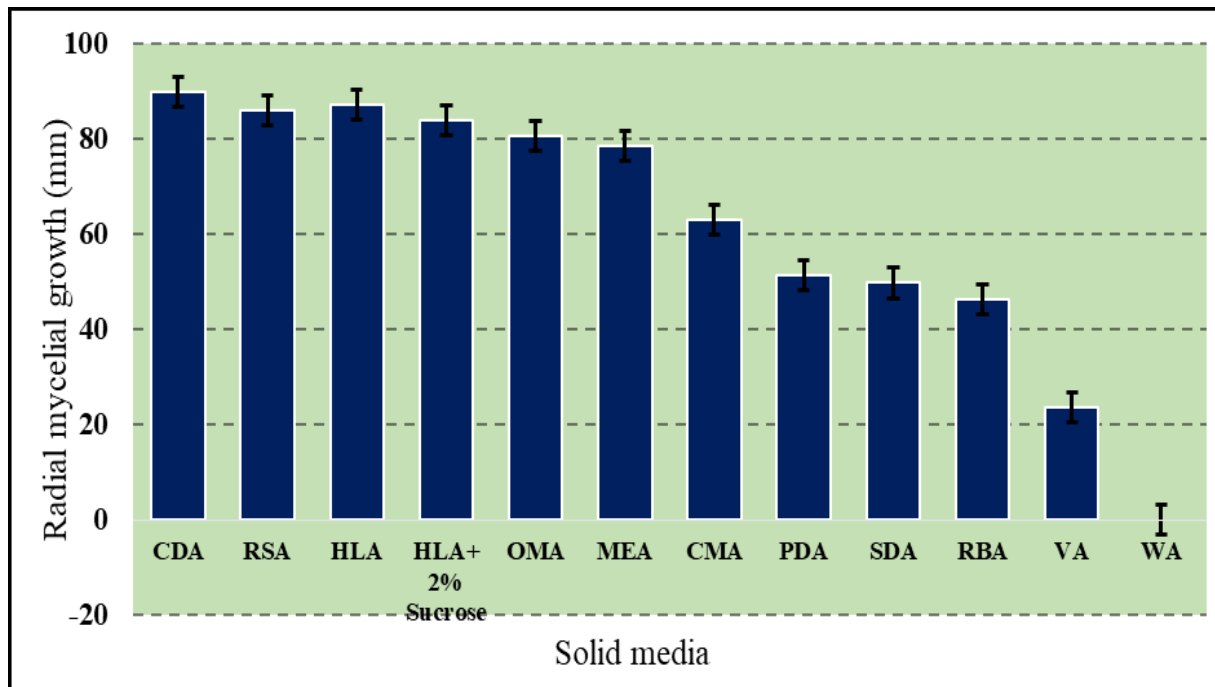


Fig 1: Effect of different solid media on mycelial growth of *B. setariae*



Plate 1: Effect of different media on growth and sporulation of *B. setariae*

3.2 Influence of pH on mycelial growth and sporulation in solid and liquid media

Here, both solid (PDA) and liquid media (PDB) were used to study the effect of pH on pathogen growth. Mycelial growth

was maximum at pH 7.0 in solid medium (81.88 mm) and was statistically on par at pH 7.0 and 8.0 in liquid media (0.59 and 0.60 g 100 mL⁻¹, respectively).

Table 3: Effect of hydrogen ion concentration (pH) on *B. setariae* infecting browntop millet in solid media

Sl. No.	pH	Dry mycelial weight (g 100 mL ⁻¹)*	Sporulation	No. of Cells**
1	4.0	No growth	-	-
2	4.5	0.53	+	9 (6-10) celled
3	5.0	0.45	-	-
4	5.5	0.38	-	-
5	6.0	0.30	++	8 (4-8) celled
6	6.5	0.42	+++	9 (5-9) celled
7	7.0	0.59	+	6 (4-7) celled
8	7.5	0.30	+	6 (4-6) celled
9	8.0	0.60	-	-
S.Em±				0.01
CD (P 0.01)				0.06
CV (%)				6.60

Note: * Mean of three replications; -: No sporulation; +: 1-15 conidia; ++: 16-45 conidia; +++: 46-70 conidia per microscopic field. ** Numbers in parenthesis represents cell range.

In other pH levels, there was varied growth from 0.30 – 0.53 g as dry mycelial weight and 31.00 – 76.44 mm as radial growth in liquid and solid media, respectively. The lowest mycelial growth was noticed at pH 4.0 in liquid (no growth) and solid (31.00 mm) media. In both solid and liquid media,

maximum sporulation was recorded at pH 6.5. Though, pH 8.0 showed abundant mycelial growth, sporulation was absent. However, larger size conidia (*i.e.*, 10 celled conidia) were produced in pH 4.5 (Table 3, 4; fig. 2, 3 and plate 2, 3)



Plate 2: Growth of *B. setariae* at different pH on solid media



Plate 3: Growth of *B. setariae* at different pH on liquid media

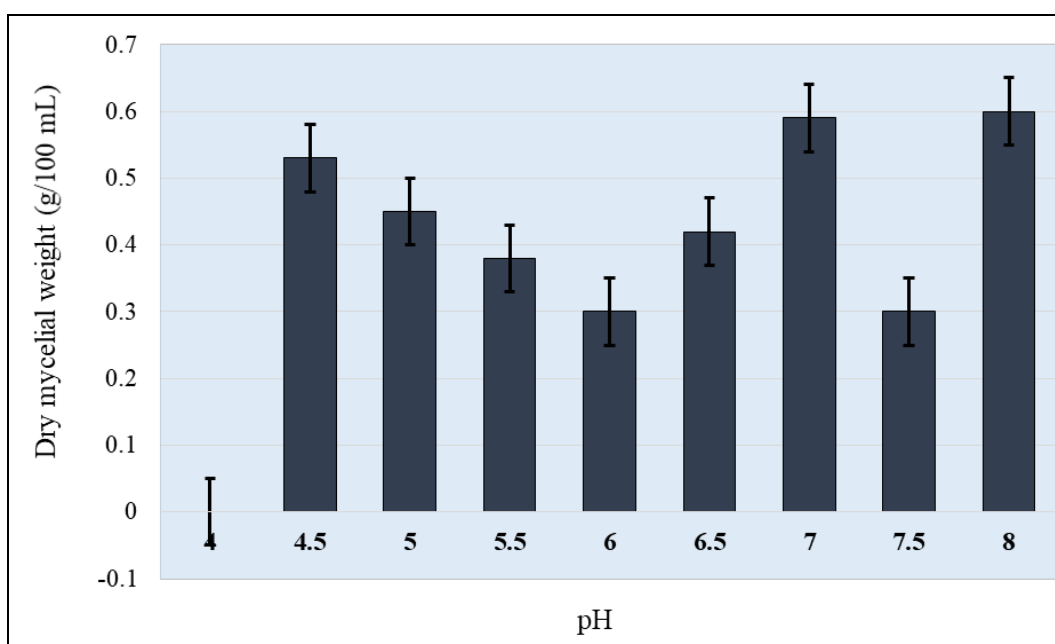


Fig 2: Effect of pH on biomass production of *Bipolaris* sp. (Liquid media)

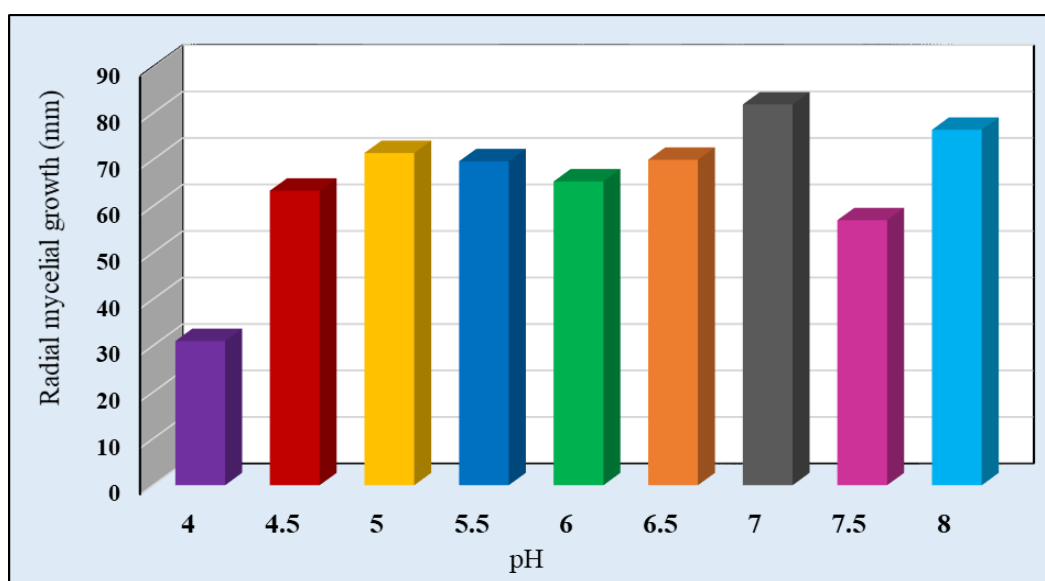


Fig 3: Effect of pH on mycelial growth of *B. setariae* (Solid media)

Table 4: Effect of hydrogen ion concentration (pH) on *B. setariae* infecting browntop millet in solid media

Sl. No	pH	Radial mycelial growth (mm)*	Sporulation
1	4.0	31.00	-
2	4.5	63.33	+
3	5.0	71.44	+
4	5.5	69.66	-
5	6.0	65.33	++
6	6.5	69.99	+++
7	7.0	81.88	+
8	7.5	56.99	-
9	8.0	76.44	+
S.Em ±		0.96	
CD (P 0.01)		1.29	
CV (%)		2.19	

Note: * Mean of three replications; -: No sporulation; +: 1-15 conidia; ++: 16-45 conidia; +++: 46-70 conidia per microscopic field.

Results showed the ideal pH to culture *B. setariae* causing browntop millet leaf blight was pH 6.0 to pH 7.0 for maximum mycelial growth and abundant sporulation. Similarly, Naz *et al.* (2012)^[9] observed the maximum growth of *H. maydis* at pH 7.0 and Sinclair (1982)^[10] noticed maximum sporulation at pH 6.5. Mishra and Prakash (1972)^[11] obtained the best growth of the *H. catenarium* at pH 6.8 and 7.2. Whereas, Campi (1939)^[12] found best growth of *H. oryzae* at pH 5.7.

Conclusion

Among the 12 media, Czapek Dox agar (CDA) and host leaf decoction agar with 2 per cent sucrose was found to be the ideal for maximum growth and sporulation of *B. setariae* infecting browntop millet. Ideal pH to culture *B. setariae* is pH 6.0 to pH 7.0 for growth and sporulation. In the liquid and solid media tested, paramount mycelial growth was observed at pH 7.0 whereas maximum sporulation was recorded at pH 6.5.

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Reference

1. Sujata B, Prabhu CG, Nandini C, Prabhakar, Thippeswamy V. Browntop Millet- A Review. Agri. Res. & Tech.: Open Access J 2018;14(5):555937.
2. Misra AP, Prakash O. *Helminthosporium* species occurring on graminaceous hosts in India. Indian J. Mycol. Pl. Pathol 1972;2:95-97.
3. Waller J, Lenne J, Waller S. Plant pathologists' pocketbook, Edn 3. CABI publishing, Wallington, UK. 2001, 528.
4. Aneja KR. Experiments in microbiology, plant pathology, tissue culture and mushroom production technology, Edn 3, New age International publishers 2001.
5. Sinijadas K, Dhar A, Bhaumik P, Chowdhury AK. Evaluation of culture medium for the growth of *Bipolaris sorokiniana* the causal agent of spot blotch of wheat. Int. J. Curr. Microbiol. App. Sci 2018;7(10):579-583.
6. Rangaswamy G, Pandurangan SV. Studies on the *Helminthosporiose* of some graminaceous hosts in south India. Journal of Annamalai University 1962;24:133-155.

7. Tanaka H. The activity of some carbohydrates produced by *C. miyabeanus*, the causal fungus of *Helminthosporium* leaf spot of rice plants. Ann. Phytopath. Soc 1956;30(4):192-195.
8. Nayak MS, Hiremath SV. Cultural, morphological and molecular characterization of *Bipolaris oryzae* causing brown leaf spot of rice in Northern Karnataka. J. Pharmacogn. Phytochem 2019;8(2):1235-1239.
9. Naz I, Sehar S, Perveen I, Rehman A, Hameed A, Ahmad Y *et al.* Optimization of cultural studies for *Cochliobolus heterostrophus* isolates from infected maize plants from different agricultural zones of Pakistan. Br. Microbiol. Res. J 2012;2(4):233-242.
10. Sinclair JB. Compendium of soybean diseases. American Phytopathological Society 1982, 27-28.
11. Mishra AP, Prakash D. Studies on new leaf blight of barley caused by *Helminthosporium catenarium*. Indian Phytopath 1972;24:803.
12. Campi MD. *H. turcicum* in Argentina republic *Lilloe*. Rev. Bot. Tucuman 1939;4:532.