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Effect of different Media, pH Levels and Temperature on Growth and Sporulation of *Microdochium sorghi* causing Zonate Leaf Spot of Sorghum

Banothu Chandrashekar*, Yogendra Singh, B.K. Namriboi, Senthil Kumar S. and Marjit Chandam
Department of Plant Pathology,
G.B. Pant University of Agriculture and Technology, Pantnagar (Uttarakhand), India.

(Corresponding author: Banothu Chandrashekar)*
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ABSTRACT: Sorghum (*Sorghum bicolor* L. Moench) is the 5th major cereal crop, used as a food, feed, fiber, and bioenergy production. Among the diseases affecting sorghum, Zonate leaf spot caused by *Microdochium sorghi* has been considered a minor issue in recent decades. However, due to the impact of climate change, this pathogen has begun to cause economic damage to both forage and grain sorghum crops. To develop effective management strategies against this disease, it is crucial to understand the cultural characteristics of the pathogen and how it responds to various physiological and temperature conditions. This study investigates the *in vitro* effects of different culture media, pH levels, and temperature on the mycelial growth and sporulation of *M. sorghi*. Among the 10 culture media examined, the pathogen displayed the highest mycelial growth on Oat Meal Agar (with a diameter of 90mm), followed by Potato Dextrose Agar (85.3 mm), Rye Agar Type-A (82.1 mm), and Corn Meal Agar (77.3 mm) while the least growth was observed on L- Asparagine Media (31.6mm). Furthermore, experiments were conducted using Oat Meal Agar to evaluate the influence of different temperature (15°C, 20°C, 25°C and 30°C) and pH levels (5.0, 5.5, 6.0, 6.5, 7.0, 7.5 and 8.0). The most optimum condition for promoting *M. sorghi* growth (90mm) were identified at pH 6.5 and pH 7.0 both at temperature 30°C. Notably, these conditions were also associated with a significant enhancement in sporulation.

Keywords: Media, *Microdochium sorghi*, pH, Sorghum, Sporulation, Temperature, Zonate.

INTRODUCTION

Sorghum [*Sorghum bicolor* (L.) Moench] is a diploid photosynthesis efficient C4 crop. It is known as king of millet or great millet due to its large grain size and is believed to have originated in Africa. Sorghum is cultivated as a multipurpose crop *viz*., staple food, animal feed, and increasingly important source of biomass for cellulosic ethanol production. Sorghum stands 5th important cereal crop globally, covering an area of around 41.04 million ha across six continents with total global production of approximately 60.38 million tons in 2021 (FAO, 2021). India ranked 2nd in sorghum area and 4th in production in the world during 2020-21 (USDA, 2021). Despite its rich genetic diversity and adaptability to adverse conditions such as abiotic and biotic stresses, sorghum suffers much from diseases which limits the productivity and account for huge losses for the farmers. Important diseases constraining global sorghum production are stalk rot, downy mildew, grain mold, rust, head smut, leaf blight, anthracnose and zonate leaf spot (Das *et al*., 2016; Mengistu *et al.,* 2018). Among the above diseases, zonate leaf spot of sorghum caused by *Microdochium sorghi* (Bain and Edgerton ex Deighton) U. Braun, 1995, Syn: *Gloeocercospora sorghi* is polycyclic and distributed throughout the wet sorghum growing regions of the world and it was first reported as a pathogen of sweet sorghum in Louisiana (Sharma *et al*., 1980).

The *M. sorghi* overwinters as a sclerotia on infected plant tissues. The soil borne sclerotia germinate and the rain-splashed conidia are dispersed to initiate the infection process (Odvody and Madden 1984). The symptoms are formed on seedling, leaf, leaf sheath and peduncle of sorghum. The first visible symptoms are the appearance of small non-diagnostic lesions on the lower leaves, which later become circular or target shaped and turn into large purple-red or dark brown lesions with 2-8 rings. Semi-oval shaped lesions occur along the leaf margin or near the midrib. In the advanced stages, dark-red to blackish purple or brown lesions on leaves and leaf sheaths coalesce, and the entire area get blighted. The sporodochia of *M. sorghi* are slimy, salmon-coloured masses on the upper surface of the blotch with dead, greyish tan tissue spotted with black specks of spherical sclerotia in a linear arrangement. Severely infected seeds showed black oval spots on the seed surface. Morphological and cultural characters of the pathogen should be diagnosed and identified for successful management of the disease.

Environmental factors such as temperature, water activity and pH have a great influence on fungal

development (Yadav *et al.*, 2014). Variation in the type of carbon and nitrogen sources besides changes in pH, temperature, incubation period, shaking and inoculum size have great influence on the growth of pathogen (Dubey, 2016). The present work depicts the role of different media, pH levels and temperature to understand ecological survival of the pathogen in the field which will be helpful in the development of management strategy for the pathogen.

MATERIALS AND METHODS

A. Isolation of pathogen

The pathogen *M. sorghi* was isolated from diseased sorghum plants collected from Livestock Research Centre, G.B. Pant University of Agriculture and Technology, Pantnagar (Uttarakhand). Sorghum leaves with typical symptoms were used for isolation. The diseased leaves were washed thoroughly in tap water and allowed to dry. After which, plant tissues with single lesion were cut into small pieces and surface sterilized with 1% sodium hypochlorite (NaClO) for 1 minute, rinsed thrice with sterile distilled water and finally dried on sterilized tissue paper under aseptic condition. Then, the cut plant tissues were placed in Petri dishes containing Oat Meal Agar (OMA) and incubated in B.O.D incubator at 28±1°C.

B. Growth of M. sorghi on different culture media

The following 10 culture media *viz*., Oat meal agar, Potato dextrose agar, Corn meal agar, Czapek dox agar, Rye agar-A, Rye Agar-B, V8-juice agar, Malt agar, Potato carrot agar and T2 (asparagine) were used to find out the most suitable medium for the mycelial growth and sporulation. Each culture medium was prepared in 1 liter of distilled water and autoclaved at 121.6°C at 15 psi for 20min. These were cooled to 45°C and then poured in 90mm Petri plates for solidification.

Medium	Composition and Quantity
Oat Meal Agar (OMA)	Rolled Oats (30g) and Agar (15g)
Potato Dextrose Agar (PDA)	Peeled and sliced potato (200g); Dextrose (20g) and Agar (20g)
Rye Agar A (Rye-A)	Rye (60g); Sucrose (20g) and Agar (15g)
Corn Meal Agar (CMA)	Cornmeal (20g); Peptone (20g); Glucose (20g) and Agar (15g)
Rye Agar-B (Rye-B)	Rye (60g); Sucrose (20g); Beta-sitosterol (0.050g) and Agar (15g)
Czapeks Dox Agar (CDA)	Di potassium hydrogen phosphate (1g); Sodium nitrate (2g); Magnesium sulphate (0.5g); Potassium chloride (0.5g); Sucrose (30g); Ferrous sulphate (0.01g) and Agar (20g)
Malt Agar (MA)	Malt extract powder (20g); Glucose (20g); Peptone (1.0g) and Agar (20g)
Potato Carrot Agar (PCA)	Peeled and sliced potato (300g); Carrot (25g) and Agar (15g)
V-8 Juice Agar (V-8)	V-8 juice (100 ml); L-Asparagine (10g); Yeast extract (2g); Calcium carbonate (2g); Glucose (2g) and Agar (20g)
T-2 Agar (T-2)	L- Asparagine (10g); Sucrose (100g); Yeast extract (0.1g); Potassium dihydrogen phosphate (0.25g); Magnesium Sulfate (0.25g); Ferrous sulphateheptahydrate (0.02g); Zinc Sulphateheptahydrate (0.015g); Potassium chloride (0.12g); Calcium Nitrate Tetrahydrate (1.0g) and Agar (20g)

C. Growth of M. sorghi on different pH levels

The impact of pH was examined using a range of 7 pH levels from 5.0 to 8.0, with a difference of 0.5 between each pH level. The pH was adjusted by adding HCl or NaOH using a pH meter before autoclaving the OMA media. Each pH level was replicated thrice.

D. Growth of M. sorghi on different temperature

The Petri plates poured with sterilized OMA media were inoculated with a 5 mm mycelium disc of *M. sorghi* and incubated at temperature ranging from 15 to 30°C with a difference of 5°C between each temperature. The linear growth of the mycelium was measured at 48-hours interval. At 10 days after incubation (DAI), mycelial growth, colour of colony, growth pattern, presence and absence of microsclerotium were recorded. Spore count was recorded using a haemocytometer.

RESULTS

A. Isolation of pathogen

Microdochium sorghi was isolated from the diseased leaves of sorghum plants collected from Livestock research Centre, G.B. Pant University of Agriculture and Technology, Pantnagar (Uttarakhand). This isolate was brought into axenic culture. The pathogen was identified at molecular level with the accession number OR164443 (NCBI GenBank).

B. Growth of M. sorghi on different culture media

In the study, a total of 10 different media were employed to investigate the growth of *M. sorghi*. The results, as presented in Table 1, revealed important findings regarding the optimal conditions for the growth and sporulation of the test pathogen. The maximum colony diameter of 90.0 mm was recorded on Oat Meal Agar (OMA). The next best medium for *M. sorghi* growth was Potato Dextrose Agar, which showed a colony diameter of 85.3 mm, followed by Rye Agar Type-A with 82.1 mm **(**Plate 1). While the lowest colony diameter, measuring 31.6 mm, was observed in the L-Asparagine medium. Moreover, the test pathogen showed sporulation in 6 out of the 10 media tested *viz*., Oat Meal Agar, Potato Dextrose Agar, Rye Agar Type-A, Rye Agar Type-B, Malt Agar Media and V8-Juice Agar. However, the most excellent sporulation was observed in Oat Meal Agar, indicating that it was the most conducive medium for *M. sorghi* growth and sporulation. Sclerotial bodies were present in all test media, but abundant sclerotia were observed on OMA.

Table 1: Effect of different media on mycelial growth of *M. sorghi.*

Sr. No.	Medium	Colony diameter (mm)	Cultural Characteristics				Sporulation	Sclerotia
			Colour	Elevation	Margin	Zonation		
1.	Oat Meal Agar	90.0	Salman pink	Sub-raised	Entire	Present	+++	+++
2.	Potato Dextrose Agar	85.3	Grey	Flat	Irregular	Absent	+	++
3.	Rye Agar Type-A	82.1	Salman pink	Raised	Entire	Present	++	++
4.	Corn Meal Agar	77.3	Light brown	Flat	Undulated	Present	--	+
5.	Rye Agar Type-B	60.8	Salman pink	Raised	Entire	Present	+	+
6.	Czapek Dox Agar	52.3	White	Sub-raised	Entire	Absent	--	+
7.	Malt Agar Media	45.4	Grey	Flat	Undulated	Present	+	-
8.	Potato Carrot Agar	35.3	Light brown	Sub-raised	Undulated	Present	--	+
9.	V8-Juice Agar	34.5	Whitish grey	Sub-raised	Irregular	Present	+	+
10.	L- Asparagine (T-2) Media	31.6	Salman pink	Flat	Irregular	Present	--	+
	C.D at 5%	3.8						
	C.V	1.3						
	SE(m)	2.4						

*Average of three replication

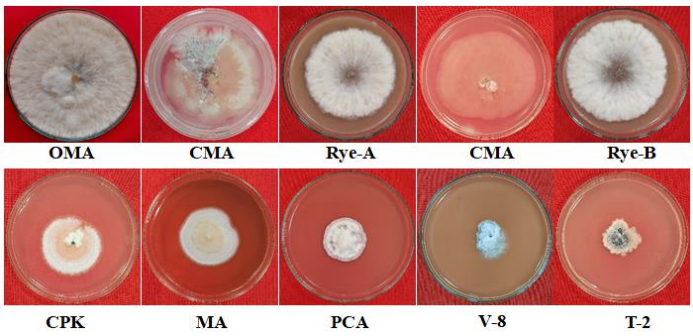

Plate 1. Effect of different media on mycelial growth of *M. sorghi.*

C. Growth of M. sorghi on different pH and Temperature

Subsequently, a further experiment was conducted on Oat Meal Agar to determine the optimal conditions for the growth of *M. sorghi* at 5 different temperature and 7 pH levels. The findings showed that sporulation of *M. sorghi* occurred at all temperature and pH levels tested, except for pH 5.0, where sporulation was not observed (Table 2). The highest radial growth (90.0 mm) of the pathogen was achieved at pH 6.0 and pH 6.5, at 10 days after incubation (DAI) at 30°C followed by pathogen radial growth at pH 7.0 (84.0 mm) and pH 7.5 (71.0 mm) at temperature 30°C (Table 2). But the smallest colony diameter was observed at pH 8.0 (17.3 mm) after 10 DAI at 15°C (Plate 2). The study demonstrated that the growth of the test pathogen was negatively affected as the pH level deviated from the range of 6.0 to 6.5. Extreme acidic and alkaline pH levels as well as temperature below 30°C were found to be unsuitable for the growth and sporulation of *M. sorghi.*

Table 2: Effect of different temperatures and pH levels on growth of *M. sorghi* on Oat Meal Agar.

pH / Temp. (°C)	Colony diameter (mm)				Sporulation	Sclerotia
	15	20	25	30		
5.0	21.0	39.3	43.7	51.7	--	+
5.5	21.3	40.7	65.7	67.0	+	+
6.0	21.7	44.3	74.7	90.0	++	+
6.5	23.3	50.3	77.3	90.0	+++	+
7.0	14.3	52.3	74.7	84.0	++	+
7.5	21.3	42.3	70.3	71.0	+	+
8.0	17.3	44.7	58.3	63.3	+	+
C.D at 5%	1.9	1.4	2.3	1.5		
C.V	4.1	1.9	2.4	0.5		
SE(m)	0.6	0.5	0.8	1.4		

*Average of three replication

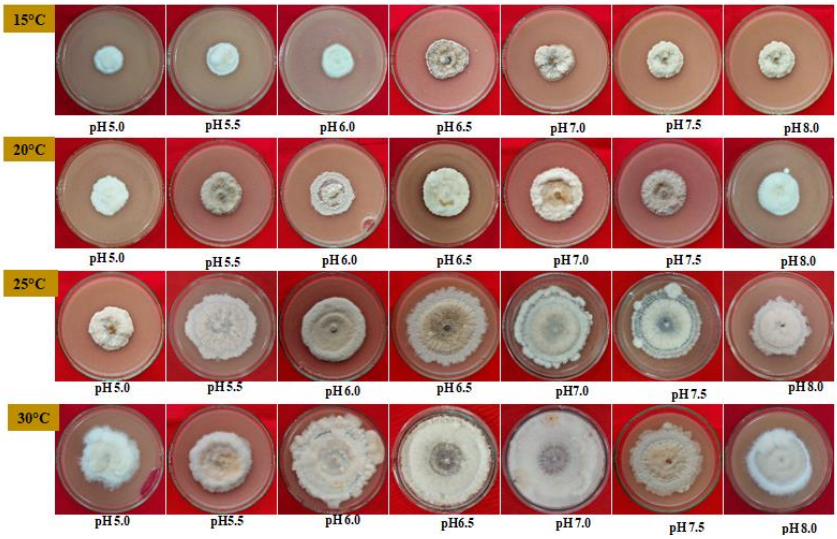

Plate 2. Effect of different temperature and pH levels on growth of *M. sorghi* on Oat Meal Agar.

CONCLUSIONS

Microdochium sorghi isolated from diseased sorghum leaves exhibited varying growth responses to different culture media, pH levels and temperature. Oat Meal Agar (OMA) emerged as the most suitable medium for *M. sorghi* growth, yielding a substantial colony diameter of 90.0 mm. Remarkably, OMA also provided the most conducive conditions for sporulation. Furthermore, experiments on OMA highlighted that pH levels of 6.0 and 6.5, combined with a temperature of 30°C, were optimal for achieving a maximum colony diameter of 90.0 mm. Deviations from this pH range with extreme acidity and alkalinity, as well as temperatures below 30°C were unfavorable for *M. sorghi* growth and sporulation. These findings will provide valuable insights into the culturing and management of *M. sorghi*.

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Conflict of Interest. None.

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