Research Article

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In-vitro study of different solid media, pH and temperature on mycelial growth and sporulation of *Alternaria alternata* causal of *Alternaria* leaf blight of bael

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ABSTRACT

Among the seven solid media, potato dextrose agar (PDA) exhibited the highest radial mycelial growth, with a pH range of 4.5-7.5 being optimal, particularly at pH 6.0 for both growth and sporulation. Richard's agar also favored significant growth *Alternaria alternata* causal of alternaria leaf blight of Bael. Temperature trials conducted at 15°C, 20°C, 25°C, 30°C and 35°C revealed that 25°C was the optimal temperature for mycelial growth and sporulation. Significant reductions in growth and sporulation were observed at temperatures below 20°C and above 30°C. These findings provide valuable insights into the ecological preferences of *A. alternata*, contributing to the development of effective management strategies for Alternaria leaf blight in bael cultivation.

Introduction

The bael tree, scientifically known as *Aegle marmelos*, holds significant cultural, religious and medicinal importance in India. Belonging to the Rutaceae family, it exhibits a chromosome number of 2n=36 (n=18). Bael tree, referred to as bael in India, has been revered in Indian mythology since prehistoric times and is particularly consecrated to Lord Shiva. Its prominence is noted in ancient texts such as the Jain scriptures, Buddhist literature, the *Ramayana* and the *Yajurveda* (Singh *et al.*, 2023). Geographically, the bael tree thrives throughout most Indian states with higher concentrations in regions like Madhya Pradesh, West Bengal, Uttar Pradesh, Bihar and Odisha (Teaotia *et al.*, 1963). The bael plant is both nutritionally rich and therapeutically valuable, thriving in regions of India with limited water resources.

The fruit is a rich source of carbohydrates, vitamin A, riboflavin, alkaloids, coumarins and steroids. Marmelosin, a key bioactive compound, is believed to be highly effective in treating stomach disorders. The concentration of bael's therapeutic bioactive component, which varies based on variety (0.03-0.37%) and environmental conditions, plays a crucial role in its medicinal properties (Dixit and Dutt,

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1932). The bael plant exhibits a strong resistance to diseases with the only major documented issue being leaf spot disease caused by *Alternaria alternata* in northern India (Madaan and Gupta, 1985). Fungal infections during storage are the only significant threat to bael fruit. Alternaria leaf spots, which commonly affect the tree, are characterized by small, dark lesions (4 to 8 mm) that typically appear near the leaf margins. These lesions are often surrounded by concentric rings, further distinguishing the disease.

Material and Methods

The experiment was conducted during 2023-24 in the Laboratory of the Department of Plant Pathology, College of Agriculture, JNKVV, Jabalpur, Madhya Pradesh, India. The laboratory study aimed to evaluate the effects of various solid culture media, pH levels and temperature on the radial mycelial growth and sporulation of *Alternaria alternata*, the causal agent of Alternaria leaf blight in bael (*Aegle marmelos*). Seven different solid media *viz.,* Potato Dextrose Agar (PDA), Corn Meal Agar, Czapek's Dox Agar, Oatmeal Agar, Richard's Agar, Bael Leaf Extract Agar and Water Agar were evaluated for their effects on radial mycelial growth and sporulation of *Alternaria alternata*. The formulations of each medium were weighed and prepared accordingly, with all media sterilized prior to use. Sterilized cork borers were used to inoculate 5 mm mycelial discs into petriplates containing the sterilized media. The plates were then incubated at 25 ± 2ºC for seven days with three replications for each medium. Radial growth was assessed by measuring the colony diameter and two diagonals passing through its center, excluding the initial 5 mm disc. Mycelial growth and sporulation on the seven media were observed at regular intervals throughout the incubation period. Colony characteristics were also recorded during the assessment.

The study of varying pH levels was conducted to determine the effect of different hydrogen ion concentrations in the medium on the fungal growth of *Alternaria alternata*. The pH of the PDA medium was adjusted to 5.0, 5.5, 6.0, 6.5, 7.0 and 7.5 using N/10 NaOH to increase the pH and N/10 HCl to decrease it. After autoclaving, the medium was poured into Petri plates and inoculated with fungal cultures. The plates were incubated at 25 ± 1°C and radial mycelial growth and sporulation were recorded at regular intervals. In addition, the effect of five different temperatures (15°C, 20°C, 25°C, 30°C and 35°C) on the growth of *A. alternata* was also examined. For this, 20 ml of sterilized PDA was poured into each Petri dish. The inoculated plates were incubated at the specified temperatures with four replications for each condition. Mycelial growth and sporulation were observed and recorded after seven days of incubation. The data collected during the experiment were statistically analyzed using the methods given by Panse and Sukhatme (1995).

Results and Discussion

The radial growth of *Alternaria alternata* varied significantly across all media tested. Maximum radial growth was observed on Potato Dextrose Agar (72.6 mm), which was statistically at par with the growth on Corn Meal Agar (72.3 mm) after eight days of incubation, followed by Czapek's Dox Agar (58.0 mm) and Bael Leaf Agar (54.6 mm) (Table 1, Fig. 1 and Fig. 4). The data indicated that the highest mean radial growth occurred on Corn Meal Agar (40.25 mm), followed by Potato Dextrose Agar (36.4 mm). The lowest radial growth was recorded (Table 1) on Richard's Agar (32.2 mm), which was similar to Bael Leaf Agar (32.2 mm) with Water Agar showing the least growth (16.0 mm). In terms of sporulation, the highest number of spores per microscopic field (28) was observed on Potato Dextrose Agar, followed closely by Bael Leaf Agar (27). These findings are consistent with those reported by Hubballi *et al.* (2010), Apet *et al.* (2014), Reddy *et al.* (2019) and Solanki *et al.* (2023), which support the results of this study.

The experimental results showed that the maximum radial growth (60.6 mm) of *Alternaria alternata* was recorded at pH 6.5 followed by pH 5.5 (57.3 mm) and pH 7.0 (55.3 mm) on the 7th day of incubation (Table 2, Fig. 2 and Fig. 5). The lowest radial growth was observed at pH 4.5 (32.6 mm). The highest sporulation occurred at pH 6.5 with an average of 38 spores per microscopic field followed by pH 7.5 with 35 spores per microscopic field. The lowest sporulation was recorded at pH 6.0 with an average of 27 spores per microscopic field. These findings align with previous research conducted by Samuel *et al.* (1972), Saeed *et al.* (1995), Hubballi *et al.* (2010), Madhavi *et al.* (2012), Ramjegathesh and Ebenezar (2012), Gholve *et al.* (2017), Gayithri *et al.* (2021), Kurhade *et al.* (2021), Mahalakshmi *et al.* (2021) and Fagodiya *et al.* (2022).

The results indicated that a temperature of 25ºC was optimal for the radial growth of *Alternaria alternata* with a maximum growth of 73.7 mm. Temperatures of 30ºC and 20ºC also supported significant radial growth, measuring 50.7 mm and 41.2 mm, respectively (Table 3, Fig. 3 and Fig. 6). The temperature range of 20–30ºC was found to be most favorable for fungal growth. In terms of sporulation, the highest number of spores (35 per microscopic field) was observed at 25ºC. Sporulation was also substantial at 20ºC and 30ºC with 27 and 30 spores per microscopic field, respectively. These findings are consistent with the reports of Waghunde and Patil (2010), Gholve *et al.* (2017), Kurhade *et al.* (2021), Mahalakshmi *et al.* (2021) and Fagodiya *et al.* (2022).

Table 1. Effect of solid media on growth of *A. alternata*

S.No.	Media	Radial growth (mm) after (days)								Mean	Spores per microscopic field	Index
		1	2	3	4	5	6	7	8			
1.	Potato Dextrose Agar	15.0	20.6	23.0	29.3	34.3	40.0	57.0	72.6	36.4	28	Very good
2.	Corn Meal Agar	13.6	21.3	24.3	36.0	41.6	51.3	61.3	72.3	40.25	19	Good
3.	Czapek's Dox Agar	14.0	20.3	24.0	30.6	36.3	42.6	49.6	58.0	34.4	20	Very good
4.	Oat Meal Agar	14.0	26.6	32.0	39.6	43.6	48.0	43.0	44.0	36.3	17	Good
5.	Richard's Agar	10.3	17.3	23.6	31.0	35.3	45.0	51.0	44.6	32.2	23	Very good
6.	Bael Leaf Agar	14.3	22.6	23.6	27.6	32.6	38.0	45.0	54.6	32.2	27	Very good
7.	Water Agar	6.6	8.3	11.0	13.3	16.0	21.3	24.3	27.6	16.0	03	Fair
SEm±		0.7	0.9	0.8	0.9	0.9	2.0	0.7	0.6	0.6		
CD (p=0.05)		2.1	2.7	2.6	2.9	2.7	0.6	2.2	1.9	1.8		

Table 2. Effect of pH on growth of *A. alternata*

S.No.	pH	Radial growth (mm) after (days)							Mean	Spores per microscopic field	Index
		1	2	3	4	5	6	7			
1.	4.5	11.1	15.6	22.3	27.3	31.0	32.6	32.6	25.6	33	Excellent
2.	5.0	12.0	20.0	24.6	27.8	34.0	36.0	52.5	32.7	33	Excellent
3.	5.5	11.3	15.3	19.6	25.6	31.6	36.0	57.3	31.8	28	Very good
4.	6.0	14.0	17.3	22.0	26.3	33.3	36.0	51.6	31.6	27	Very good
5.	6.5	8.6	17.6	25.3	31.0	43.6	45.6	60.6	37.2	38	Excellent
6.	7.0	11.0	16.6	21.3	25.0	35.0	37.3	55.3	32.5	29	Very good
7.	7.5	8.3	14.6	17.6	20.3	25.0	25.3	46.0	25.5	35	Excellent
SEm±		0.6	0.9	0.6	0.9	0.9	0.7	0.9			
CD (p=0.05)		2.1	2.7	2.1	2.9	2.7	2.4	2.7			

Table 3. Effect of temperature on growth of *A. alternata*

S.No.	Temperature (°C)	Radial growth (mm) after (days)							Mean	Spores per microscopic field	Index
		1	2	3	4	5	6	7			
1.	15	18.7	24.0	27.5	32.75	36.0	37.0	37.7	30.6	6	Fair
2.	20	14.0	19.5	24.2	29.0	33.2	36.0	41.2	28.1	27	Very good
3.	25	13.2	22.2	33.0	43.5	61.2	65.0	73.7	44.5	35	Excellent
4.	30	11.7	18.0	25.5	31.25	35.5	42.0	50.7	30.9	24	Very good
5.	35	14.2	17.2	20.7	24.75	27.7	27.0	29.5	23.0	16	Good
SEm±		0.6	0.5	0.7	0.6	0.7	0.6	0.9			
CD (p=0.05)		1.8	1.6	2.3	2.0	2.2	1.9	2.9			

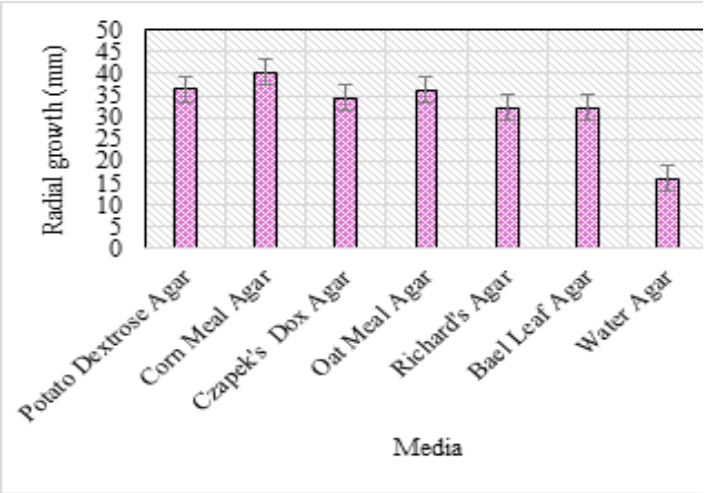

Fig. 1. Effect of solid media on radial growth of *A. alternata*

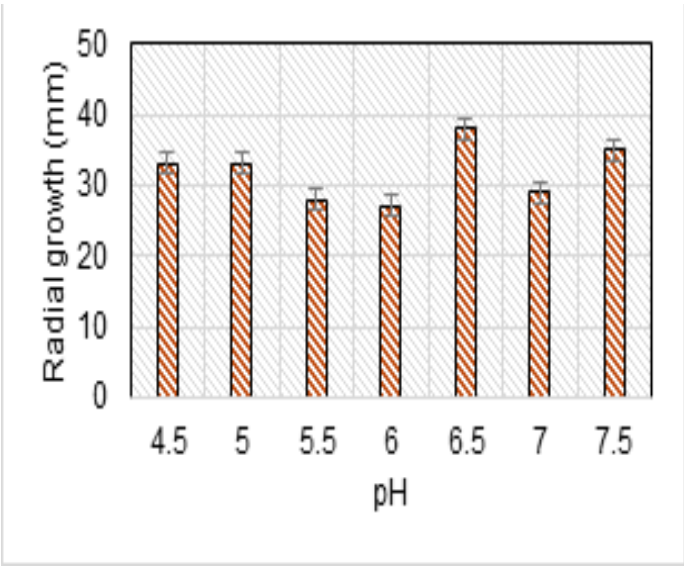

Fig. 2. Effect of pH on radial growth of *A. alternata*

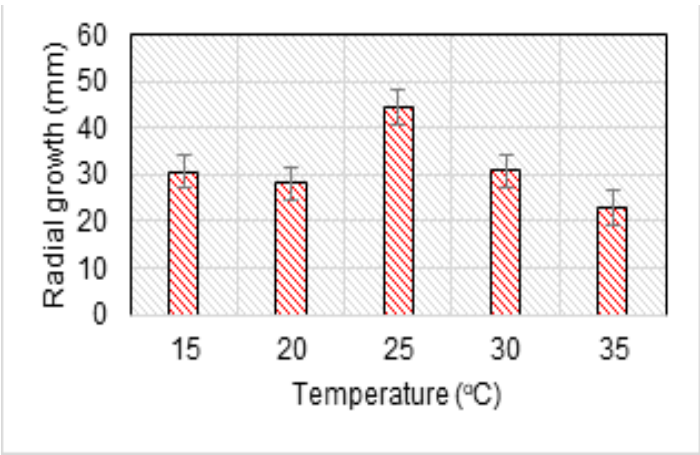

Fig. 3. Effect of temperature on radial growth of *A. alternata*

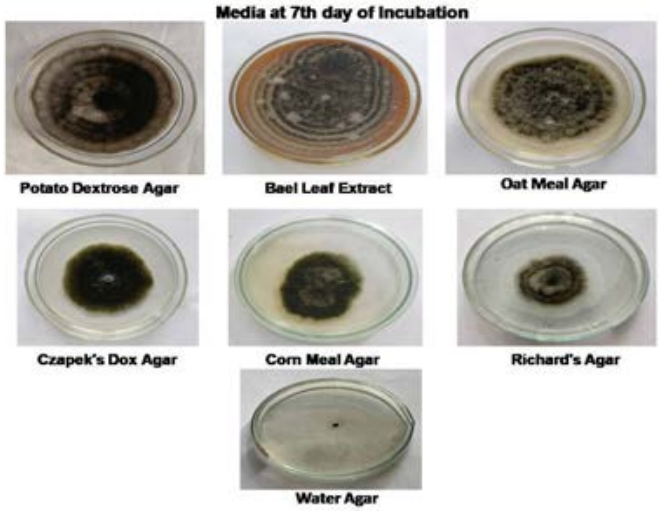

Fig. 4: Effect of solid media on growth of *A. alternata*

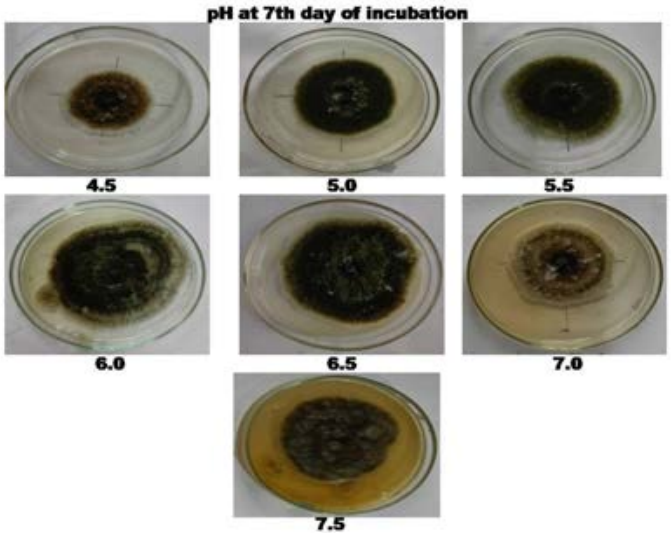

Fig. 5: Effect of pH on growth of *A. alternata*

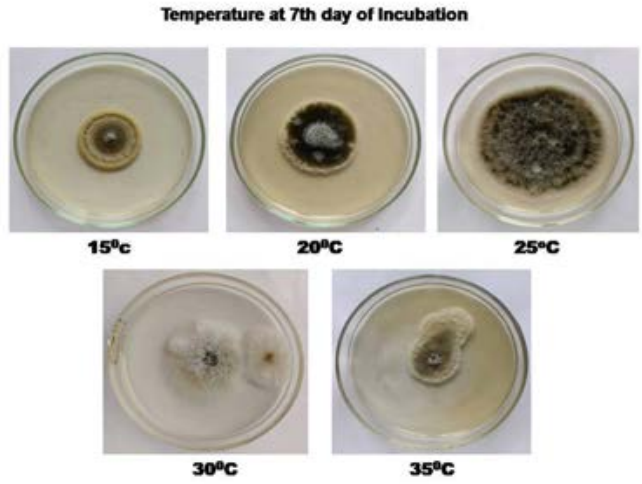

Fig. 6: Effect of temperature on growth of *A. alternata*

Conclusion

The study highlighted the impact of varying growth conditions *i.e.*, media type, pH and temperature-affect *Alternaria alternata*'s radial growth and sporulation. Potato Dextrose Agar (PDA) and Corn Meal Agar (CMA) promoted optimal mycelial growth, while PDA and Bael Leaf Agar enhanced sporulation, indicating nutrient-specific influences on fungal reproduction. Optimal growth occurred at pH 6.5, which maximized both mycelial expansion and spore production, whereas acidic conditions (pH 4.5) severely inhibited fungal development. Temperature played a critical role with 25°C identified as ideal for growth and sporulation. Though growth persisted at 20°C and 30°C, extreme deviations from the optimal temperature reduced fungal performance. These findings underscore the importance of environmental factors in *Alternaria alternata*'s physiology, offering insights for managing its proliferation in agricultural settings. The results suggest tailored strategies, such as adjusting pH or temperature in storage environments, could mitigate fungal growth. Future studies should explore molecular pathways underlying these responses to advance targeted disease control methods, potentially reducing reliance on chemical treatments.

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Conflict of Interest

The authors have no conflict of interest.

Data Sharing

All relevant data are within the manuscript.

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