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In vitro Evaluation of pH and Temperature on Mycelial Growth and Sporulation of *Exserohilum turcicum* causing Turcicum Leaf Blight of Maize

Akash Bevanur[1]*, B. Manjunath[2], Yashwantha P.[1] and N. Mallikarjuna[3]
[1]Department of Plant Pathology, UAS, Bangalore-560065 (Karnataka), India.
[2]ICAR-AICRP on Pigeonpea, ZARS, UAS, Bangalore-560065 (Karnataka), India.
[3]ICAR- AICRP on Maize, ZARS, V. C. Farm, Mandya-571405 (Karnataka), India.

(Corresponding author: Akash Bevanur)*
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ABSTRACT: Turcicum leaf blight (TLB), caused by the fungal pathogen *Exserohilum turcicum*, is a destructive foliar disease of maize that leads to considerable yield reduction across major maize-producing regions worldwide. The growth of the pathogen and subsequent disease development are strongly affected by environmental conditions, especially temperature and pH. Therefore, the present investigation was undertaken to evaluate the effect of different pH ranges and temperature conditions on the mycelial growth of *E. turcicum* under laboratory conditions. Potato Dextrose Agar (PDA) medium was adjusted to seven pH levels (4.5, 5.0, 5.5, 6.0, 6.5, 7.0 and 7.5) and inoculated with a 5 mm mycelial disc obtained from an actively growing culture. For temperature studies, inoculated plates were incubated at 15°C, 20°C, 25°C, 30°C, and 35°C. Maximum radial growth of the fungus was observed at pH 6.5, whereas reduced growth occurred at both lower (4.5) and higher (7.5) pH values. Regarding temperature, optimal growth was recorded at 25°C, followed by 30°C, while minimal growth was noted at 15°C and 35°C. These findings suggest that *E. turcicum* favors slightly acidic to near-neutral pH conditions and moderate temperatures, which has important implications for understanding disease development and designing effective management strategies.

Keywords: Maize, *Exserohilum turcicum*, Turcicum leaf blight, pH, temperature, *in vitro*.

INTRODUCTION

Maize (*Zea mays* L.) is one of the world's most widely produced cereal crops, providing food, animal feed, and raw materials for many industries (FAO, 2022). Maize is a key crop in the Indian agricultural system due to its high productivity and ability to thrive in a variety of agro-climatic conditions (ICAR, 2021).

The disease causes elongated, cigar-shaped necrotic lesions on the leaves, resulting in lower photosynthetic efficiency and resultant yield losses (Agrios, 2005). Environmental parameters including temperature, humidity, and pH have a impact on disease development and severity, as they directly affect pathogen growth, survival, and infection processes (Pandurangegowda *et al*., 2018).

A good understanding of the pathogen's physiological requirements is critical for disease prediction and successful disease management (Singh *et al*., 2020). As a result, the current study was conducted to determine the influence of various pH levels and temperature regimes on the growth of *E. turcicum in vitro*.

MATERIALS AND METHODS

A. Isolation and Maintenance of the Pathogen

Leaf samples of maize exhibiting characteristic Turcicum leaf blight lesions were collected from naturally infected fields for pathogen isolation. Portions from the advancing margins of infected tissues were cut into small thin pieces and disinfected using a 0.1% sodium hypochlorite solution for 30 seconds, followed by rinsing with sterile distilled water to remove surface contaminants. The treated tissue segments were air-dried on sterile filter paper under aseptic conditions and aseptically transferred onto Potato Dextrose Agar (PDA) plates. The plates were incubated at 25 ± 2 °C to promote fungal colonization. The emerging fungal growth was purified through the hyphal tip isolation technique, and the cultures were preserved on PDA slants for use in subsequent experimental studies.

B. Effect of pH on Mycelial Growth

Prior to autoclaving, the pH of the PDA medium was adjusted to the required levels (4.5, 5.0, 5.5, 6.0, 6.5, 7.0, and 7.5) using 0.1 N hydrochloric acid or 0.1 N sodium hydroxide. Around 20 mL of the prepared medium was dispensed into sterile Petri dishes and allowed to solidify. A 5 mm agar plug taken from the actively growing margin of the fungal culture was placed at the center of each plate. The inoculated plates were incubated at 25 ± 2°C. Fungal colony development was evaluated after seven days by recording radial growth along two perpendicular axes and computing the average value. Each treatment was performed in three replications to maintain experimental accuracy and consistency.

C. Effect of Temperature on Mycelial Growth

To determine the influence of temperature, PDA plates were inoculated with a 5 mm mycelial disc and incubated at five different temperatures: 15°C, 20°C, 25°C, 30°C, and 35°C. After seven days of incubation, we saw radial development. All treatments were replicated three times.

RESULTS AND DISCUSSION

A. Effect of pH on Mycelial Growth

The findings revealed that pH has a substantial impact on the mycelial development of *Exserohilum turcicum*. The maximum radial growth was observed at pH 6.5, followed by pH 5.5 and pH 7.5, demonstrating that the pathogen thrives in slightly acidic to near-neutral environments. The significantly reduced growth at pH 4.5 and 7.5 indicates that extreme acidic and alkaline environments are unfavourable for fungal development. These findings are consistent with previous research demonstrating that most phytopathogenic fungi thrive in pH ranges of 5.5 to 7.0 (Agrios, 2005; Pandurangegowda *et al*., 2018).

Enhanced growth near neutral pH can be due to excellent enzyme activity, membrane integrity, and effective nutrient absorption, all of which promote fungal metabolism and hyphal extension (Griffin, 1994). Extreme pH circumstances, on the other hand, have the potential to disrupt cellular processes, limit enzymatic activities, and have a detrimental impact on spore germination and mycelial expansion. Naik *et al*. (2016) found similar findings, observing that *E. turcicum* grows best at pH levels ranging from 6.0 to 6.5.

B. Effect of Temperature on Mycelial Growth

The findings clearly demonstrated that temperature had a substantial impact on *E. turcicum* mycelial growth. The highest rate of growth was seen at 25°C, with somewhat reduced growth at 30°C and 20°C, and very limited growth at 15°C and 35°C. Based on these observations, the optimal temperature range for fungal development is 25-30°C. Previous studies have shown that moderate temperature conditions promote both growth and sporulation of *E. turcicum* (Sharma *et al*., 2015; Pandurangegowda *et al*., 2018).

Increased growth at ideal temperatures could be attributed to improved metabolic activity and enzyme efficiency, which encourage rapid hyphal elongation and biomass synthesis (Griffin, 1994). In contrast, low temperatures slow metabolic rates, but high temperatures can cause cellular stress and protein denaturation, ultimately limiting fungal development (Agrios, 2005). Harlapur *et al*. (2007) found similar results, reporting maximal mycelial growth of *E. turcicum* at 25°C in the laboratory.

Overall, the study demonstrates that pH and temperature play critical roles in controlling *E. turcicum* growth. The pathogen thrives in slightly acidic to neutral pH and mild temperature settings, which are prevalent during the maize growing season. This knowledge is essential for predicting disease outbreaks and enhancing integrated disease management techniques.

Table 1: Effect of pH on growth of *E. turcicum*.

Sr. No.	Media	1	2	3	4	5	6	7	Mean	Spores per microscopic field	Index
1.	4.5	12.1	15.94	22.65	27.95	32.56	33.51	32.6	25.33	33	Excellent
2.	5.0	12	20.54	24.6	27.8	34	38	52.5	29.92	33	Excellent
3.	5.5	11.3	15.94	19.6	25.6	31.6	36	57.3	28.19	28	Very good
4.	6.0	14	17.3	22	26.3	33.3	36	51.6	28.64	27	Very good
5.	6.5	8.95	17.68	25.3	31	43.6	45.98	60.6	33.30	38	Excellent
6.	7.0	11	16.6	21.3	25	35	37.3	55.3	28.79	29	Very good
7.	7.5	8.3	14.6	17.6	20.3	25	25.3	46	22.44	35	Excellent
	SEm±	0.69	0.91	0.63	0.97	0.95	0.71	0.91			
	CD (p=0.05)	2.10	2.79	2.84	2.95	2.98	2.69	2.79			

Table 2: Effect of temperature on growth of *E. tucicum*.

Sr. No.	Temperature (°C)	1	2	3	4	5	6	7	Mean	Spores per microscopic field	Index
1.	15	18.56	25.1	27.61	32.75	35.1	37	37.7	30.55	6	Fair
2.	20	14.82	19.5	24.2	29	33.2	36	41.2	28.27	27	Very good
3.	25	15.42	22.2	33	43.5	61.2	65	75.84	45.17	35	Excellent
4.	30	11.98	18	25.5	31.25	35.5	42	50.7	30.70	24	Very good
5.	35	14.65	17.2	20.7	24.01	28.94	27	29.5	23.14	16	Good
	S.Em±	0.65	0.51	0.73	0.65	0.75	0.69	0.95			
	CD (p=0.05)	1.80	1.65	2.35	2.05	2.24	1.95	2.84			

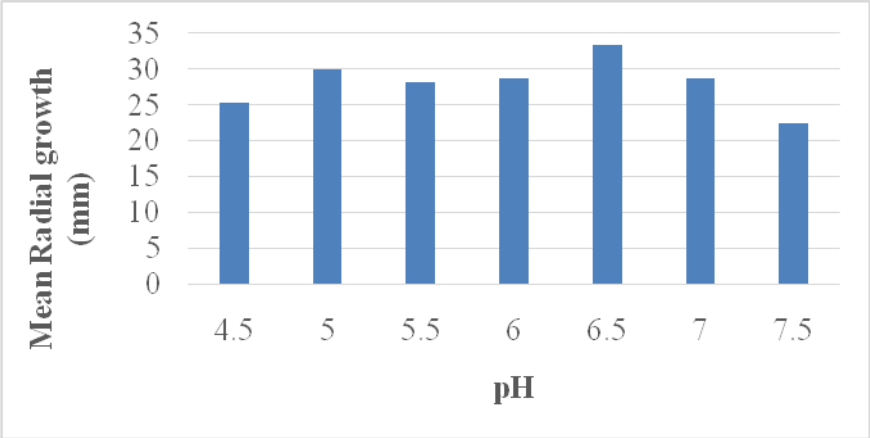


Fig. 1. Effect of pH on radial growth of *E. turcicum.*

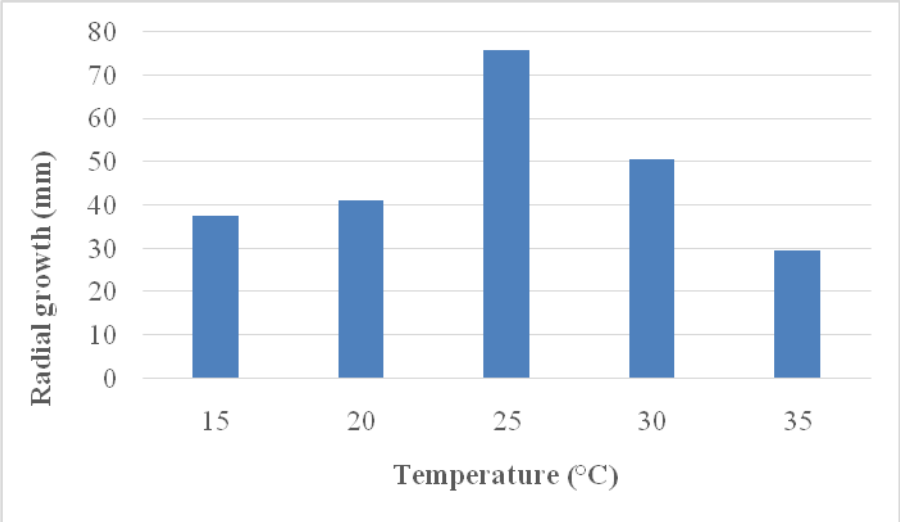


Fig. 2. Effect of temperature on radial growth of *E. turcicum.*

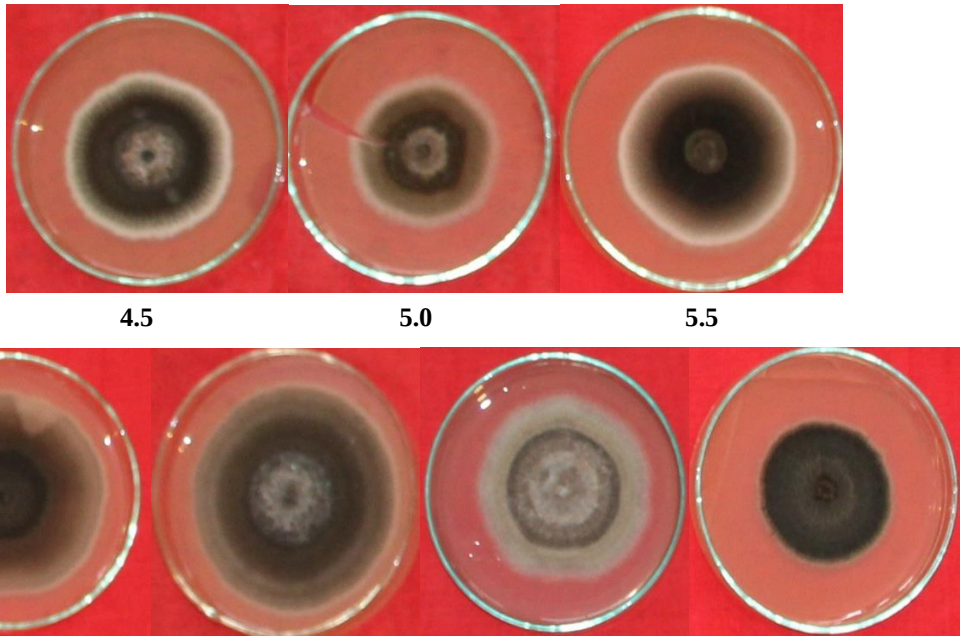
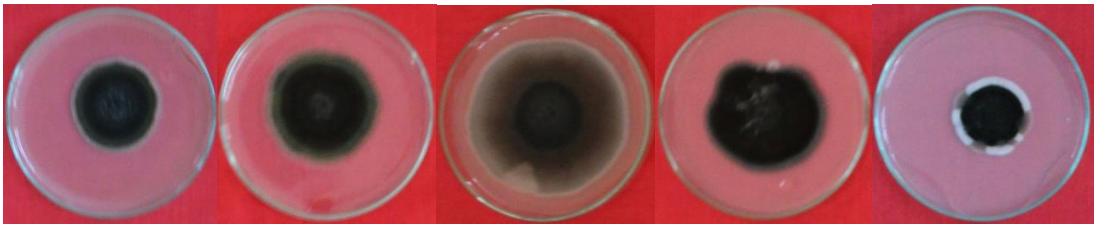


Plate 1. Effect of pH on radial growth of *E. turcicum.*



15°C 20°C 25°C 30°C 35°C

Plate 2. Effect of temperature on radial growth of *E. turcicum*

CONCLUSIONS

The results of the current work indicate that variations in hydrogen ion concentration and temperature markedly influence the growth behavior of *E.turcicum*. Maximum mycelial proliferation was observed at pH 6.5 and a temperature of 25°C, whereas elevated pH levels and higher temperatures caused a substantial reduction in fungal growth. These findings enhance understanding of the environmental requirements of the pathogen and may assist in predicting disease outbreaks as well as formulating effective management strategies for Turcicum leaf blight in maize.

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Conflict of Interest. None.

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