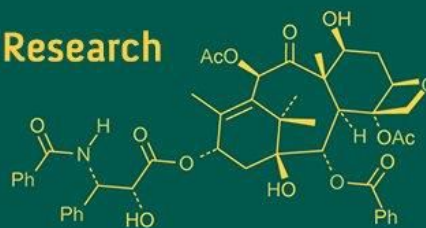
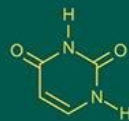
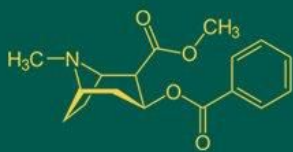


International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693
ISSN Online: 2617-4707
NAAS Rating (2025): 5.29
IJABR 2025; SP-9(8): 696-702
www.biochemjournal.com
Received: 24-06-2025
Accepted: 27-07-2025

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Cultural characterization of *Exserohilum turcicum* causing turcicum leaf blight of maize in Manipur

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DOI: <https://www.doi.org/10.33545/26174693.2025.v9.i8Sk.5236M>

Abstract

Maize (*Zea mays* L.), known as the "queen of cereals," is cultivated worldwide for its versatility, with every plant part having economic value. Turcicum Leaf Blight (TLB), caused by *Exserohilum turcicum*, is a major foliar disease limiting maize productivity. This study examined seven *E. turcicum* isolates (ET1-ET7) for their cultural and morphological variability. Colony color ranged from greyish-black to black and light greyish, though all isolates showed black pigmentation. Sporulation varied, with ET4 and ET6 being highly sporulative, while ET2 and ET5 were poor. Surface textures were rough or fluffy, and colony margins showed varying degrees of branching. Radial growth was tested on seven media, with Oatmeal Agar supporting the highest growth (7.50 cm) and Rose Bengal the least (4.18 cm). ET6 showed maximum average growth (6.57 cm), while ET2 had the lowest (3.42 cm). Growth was optimal at pH 6 and declined significantly at extreme pH levels. In liquid media, Oatmeal broth produced the highest biomass, with ET6 again being the most productive. ET2 consistently showed minimal growth and biomass. The results highlight significant variation among isolates, emphasizing the need for screening diverse maize genotypes for resistance. These findings aid in developing effective breeding strategies against TLB.

Keywords: Maize, Turcicum leaf blight, *Exserohilum turcicum*, morphological variability, radial growth, pH effect

Introduction

Maize (*Zea mays* L.) is known as queen of cereals. It is the most versatile crop every part stalk, tassel, and cob will be used to produce a large variety of food. It is grown in more than 166 countries across the globe, including tropical, subtropical and temperate regions. It is cultivated over 197 m ha with production of 1148 m tonnes with productivity of 5823.8 kg/ha all over the world. This has a wider diversity of soil, climate, and biodiversity. In India it contributes 30 per cent of the economy. Based on production maize is the third most important cereal crop in India after rice and wheat and is grown in arid to sub-humid and humid in *Kharif* season. It accounts for around 10 per cent of total food grain production in the country. It is prone to as many as 112 diseases in different parts of the world, caused by fungi, bacteria, viruses and nematodes leading to extensive damage. In India about 61 diseases have been reported to affect the crop. These include seedling blights, stalk rots, foliar diseases, downy mildews and ear rots *etc.*, (Payak *et al.*, 1973) [4]. Among the fungal diseases turcicum leaf blight caused by *Exserohilum turcicum*, (Synonyms: *Helminthosporium turcicum*) [Perfect stage: *Setosphaeria turcica*] is one of the important foliar diseases causing severe reduction in grain and fodder yield to the tune of 16-98% (Kachapur and Hegde, 1988; Chenulu and Hora 1962) [3, 1]. Turcicum leaf blight is potentially an important foliar disease in the areas where temperatures drop at night while the humidity is high. The disease is known to affect maize from seedling stage till harvest. Loss in grain yield will be more if it occurs at flowering, silking and grain filling stages. The factor affecting severity or magnitude of the disease is stage of the maize crop. Disease caused during silking stage reduces the yield drastically up to 40% (Raymond and Hooker, 1981). When the infection delays up to 6-8 weeks after silking the losses are minimum. Up to 50% of yield losses may be observed if the disease caused 2-3 weeks after pollination (Shurtleff, 1980) [9]. The grain yield reduction from 28% to 91% may be recorded because the disease.

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Methodology

Survey and collection of isolates of *E. turcicum*

The study was carried out in Imphal East, Imphal West, Bishnupur, Churachandpur districts of Manipur state of the north eastern India. Seven different pathogen samples of *E. turcicum* were collected from different places of Manipur state. For isolation of pathogen, Julius *et al.*, (2017) [2] method was followed. The diseased portion of leaf along with healthy portion of leaf was cut in to 3-5 mm size bits, surface sterilised with 1% sodium hypochlorite solution for one minute and then washed with sterile distilled water. These sterilized leaf bits were inoculated into potato dextrose agar

(PDA) aseptically and incubated for 12 days at 25 °C in the BOD incubator. The pathogen isolates were sub cultured periodically throughout the research period. These isolates were named as ET1, ET2, ET3, ET4, ET5, ET6, and ET7 and used for further studies.

Twelve days of old cultures of *E. turcicum* which were grown on PDA at 25 °C were used for recording characters like colony color, pigmentation, growth patterns and sporulation. To determine sporulation, discs of 5mm cut from the 12 days old cultures and placed in to 15ml test tube with distilled water. It was vortexed to dislodge the spores. By using hemocytometer sporulation was calculated as follows:

Table 1: Score given for sporulation (Reddy *et al.*, 2014) [7]

Score	Grade	Description
++++	Abundant	15×10 ⁴ conidia/microscopic field (40x)
+++	Good	10-15×10 ⁴ conidia/microscopic field (40x)
++	Moderate	5-10×10 ⁴ conidia/microscopic field (40x)
+	Scanty	5×10 ⁴ conidia/microscopic field (40x)

Results

Colony characters

The colony colour of the ET1, ET2, ET6 greyish black, for isolates ET4, ET5, ET7 it was black, for isolate ET3 it was light greyish. Pigmentation of all seven isolates was black. Sporulation of ET4 and et6 was Abundant. Good sporulation was produced by ET7, ET3 and ET1. Scanty sporulation was produced by ET5 and ET2.

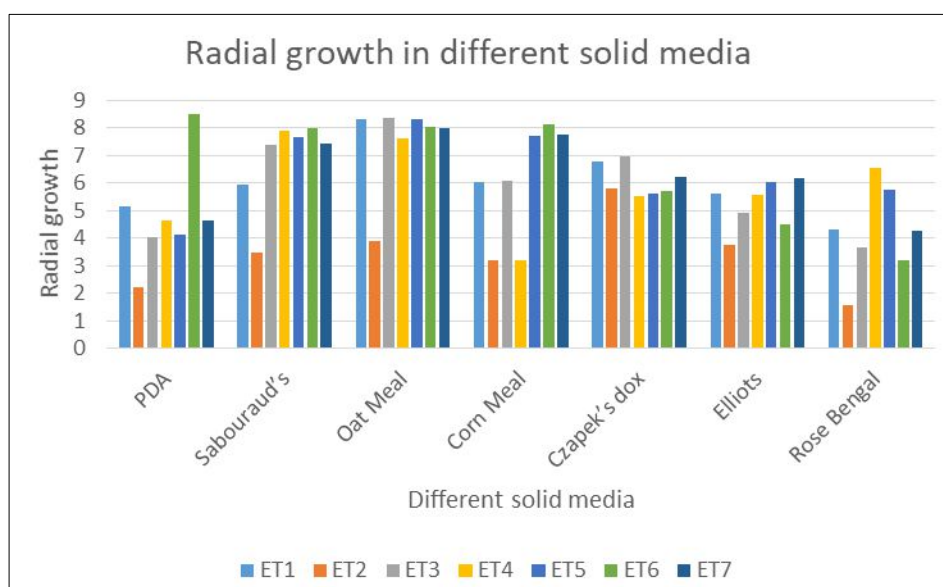
Growth patterns

There are different growth patterns in regarding surface texture. Isolates ET1, ET2, ET4, ET6 and ET7 were having rough surface texture. The surface texture of isolates ET1, ET2, ET4, ET6 were rough. Isolates ET3, ET5 the colony texture were fluffy. The colony texture of ET1, ET2, ET3 and ET6 was cottony and for isolates ET5 and ET7 it was ad pressed. And for ET3 it was distinctly velvety texture. The

edge of colony of ET1, ET4, ET5 moderately branched, isolates ET2, ET3 sparsely branched and isolates ET6, ET7 were highly branched.

Radial growth of seven different isolates of *E. turcicum* in different culture media

The radial growth of seven different isolates were studied on seven different solid culture media namely PDA, sabouraud's, oat meal agar, corn meal agar, czapek dox media, elliot's agar media and rose Bengal agar media has shown significant results ($p = 0.01$). Among all media oat meal agar was shown maximum average radial growth (7.50 cm), rose Bengal agar has recorded minimum average radial growth (4.18 cm). Among seven isolates ET6 was shown maximum radial growth (6.57 cm), Isolate ET2 was shown minimum average radial growth (3.42 cm) as shown in the graph 1.



Graph 1: Radial growth of seven different isolates of *E. turcicum* on different media

Table 2: Cultural Variations of seven different isolates of *E. turcicum*

S. No	Isolate	Incubation period for maximum Radial growth	Colony colour	Pigmentation	Sporulation	Growth pattern		
						Surface texture	Colony texture	Edge of colony
1	ET1	9	Greyish black (10Y/R 5/1)	Black	+++	Rough	Cottony	Moderately Branched
2	ET2	12	Greyish Black (10Y/R 5/1)	Black	+	Rough	Cottony	sparsely branched
3	ET3	11	Light greyish (2.5Y 5/1)	Black	+++	Fluffy	Velvety	Sparsely branched
4	ET4	8	Black (2.5 2.5/1)	Black	++++	Rough	Cottony	Moderately branched
5	ET5	10	Black (2.5 2.5/1)	Black	+	Fluffy	Ad pressed	Moderately branched
6	ET6	8	Greyish black (10Y/R 5/1)	Black	++++	Rough	Cottony	Highly branched
7	ET7	10	Black (2.5Y 2.5/1)	Black	+++	Rough	Ad pressed	Highly branched

Table 3: Radial growth (cm) of *E. turcicum* isolates in different media

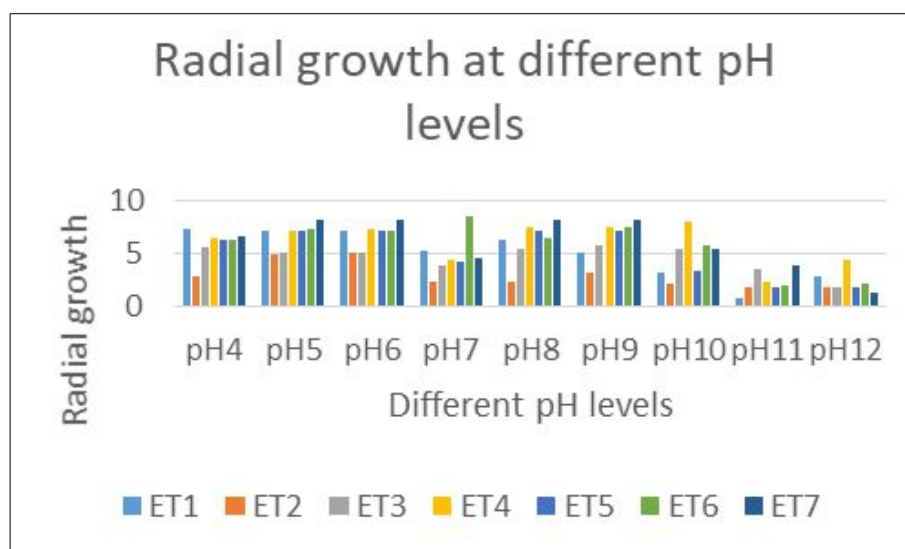
Isolate	PDA	Sabouraud's	Oat Meal	Corn Meal	Czapek's dox	Elliot's	Rose Bengal	MEAN
ET1	5.14	5.94	8.32	6.05	6.78	5.61	4.30	6.01
ET2	2.24	3.46	3.92	3.18	5.82	3.75	1.58	3.42
ET3	4.06	7.38	8.36	6.10	6.98	4.93	3.65	5.92
ET4	4.65	7.90	7.62	3.20	5.54	5.58	6.55	5.86
ET5	4.15	7.68	8.30	7.72	5.60	6.02	5.74	6.45
ET6	8.48	7.98	8.04	8.14	5.70	4.48	3.18	6.57
ET7	4.65	7.42	8.00	7.75	6.24	6.16	4.26	6.35
MEAN	4.76	6.83	7.50	6.02	6.09	5.23	4.18	

	Isolates(A)	Media(B)	Inter(A×B)
SE(m)	0.02	0.02	0.01
CD (5%)	0.057	0.057	0.037

Radial growth of seven different isolates of *E. turcicum* at different pH levels

Radial growth of seven different isolates was recorded at different pH levels namely pH4, pH5, pH6, pH7, pH8, pH9, pH10, pH11, pH12 and results was significant ($p = 0.01$). At

pH6 maximum average radial growth was recorded that was 6.76 cm, at pH12 minimum radial growth was recorded that was 1.3 cm. For isolate ET4 maximum radial growth was recorded that was 7.94 cm and for ET2 it was minimum that was 3.72 cm (Table 4 & plate 1) and as shown in the graph 2.

**Graph 2:** Radial growth of seven different isolates of *E. turcicum* at different pH levels.**Table 4:** Radial growth (cm) of seven different isolates of *E. turcicum* at different pH levels

	pH4	pH5	pH6	pH7	pH8	pH9	pH10	pH11	pH12	Mean
ET1	7.35	7.2	7.1	5.2	6.3	5.15	3.2	0.75	2.75	6.42
ET2	2.75	4.95	5.15	2.25	2.3	3.15	2.05	1.75	1.7	3.72
ET3	5.6	5.05	5.15	3.8	5.45	5.75	5.4	3.5	1.75	5.92
ET4	6.55	7.15	7.35	4.45	7.55	7.6	8.1	2.35	4.4	7.92
ET5	6.35	7.15	7.1	4.2	7.25	7.1	3.3	1.75	1.85	6.57
ET6	6.25	7.4	7.25	8.5	6.4	7.45	5.8	2	2.15	7.6
ET7	6.7	8.2	8.25	4.6	8.25	8.3	5.45	3.95	1.3	7.8
Mean	5.93	6.72	6.76	4.71	6.21	6.35	4.75	2.29	2.27	

	Isolates(A)	Media(B)	Inter(A×B)
SE(m)	0.002	0.03	0.1
CD (5%)	0.07	0.08	0.05

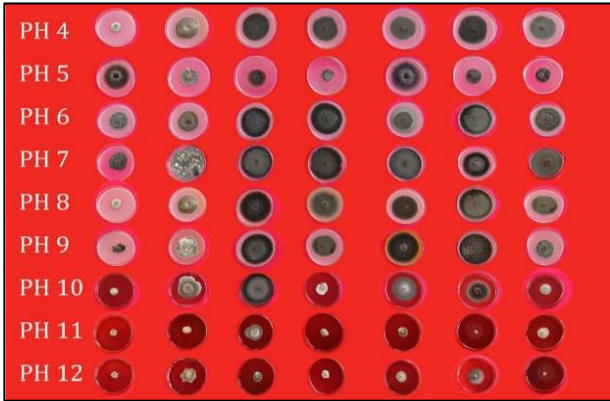


Plate 1: Radial growth of seven different isolates of *E. turcicum* at different pH levels

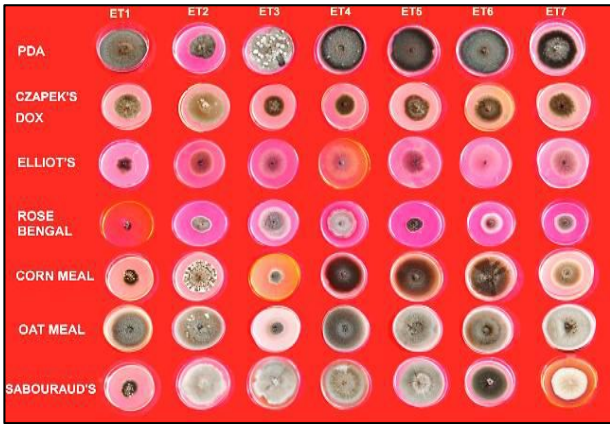
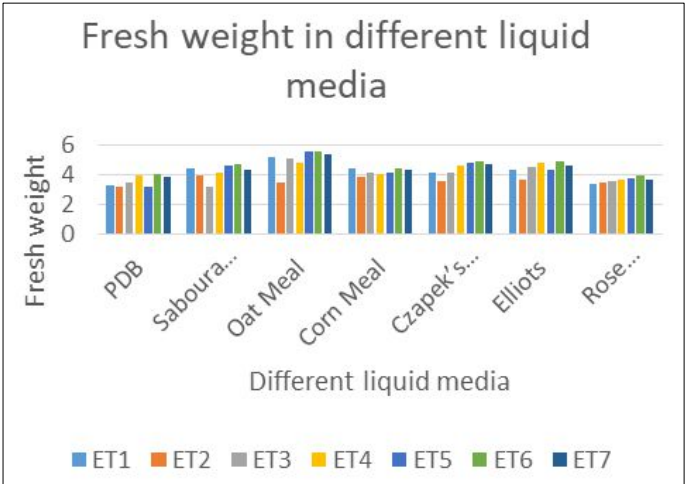


Plate 2: Radial growth of seven different isolates of *E. turcicum* on different media

Fresh weight of seven different isolates of *E. turcicum* in different liquid media

The fresh weight of mycelial mat was recorded in different liquid media namely PDB, sabouraud's, oat meal agar, corn meal agar, czapek dox media, elliot's agar media and rose Bengal agar media and the results was significant($p = 0.01$).Among all liquid media oat meal broth was recorded

maximum fresh weight that was 5.03gm and rose Bengal broth recorded minimum fresh weight that was 3.53 gm, likewise isolate ET6 was recorded maximum fresh weight that was 4.67gm and ET2 was minimum that was 3.61 gm among all isolates (Table 5) as shown in the graph 3 isolate ET6 was recorded maximum fresh weight among all isolates and oat meal broth recorded maximum fresh weight.



Graph 3: Fresh weight of seven different isolates of *E. turcicum* in different liquid media

Table 5: Fresh weight (gr) seven different isolates of *E. turcicum* in different liquid media

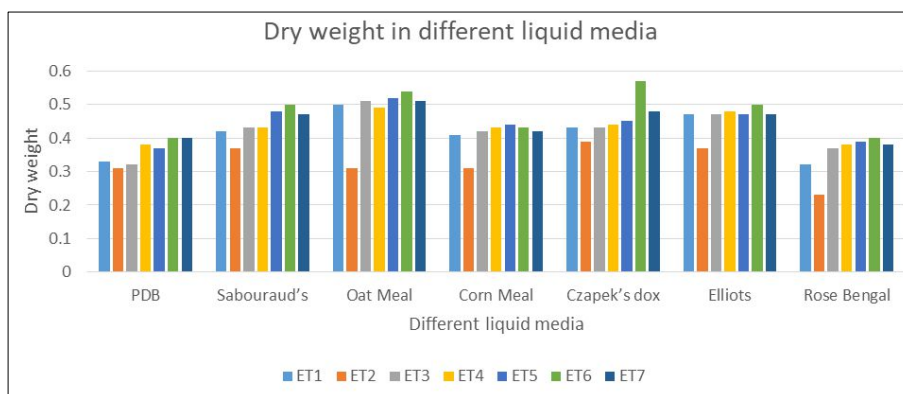
	PDB	Sabouraud's	Oat Meal	Corn Meal	Czapek's dox	Elliot's	Rose Bengal	MEAN
ET1	3.34	4.42	5.23	4.463	4.153	4.337	3.37	4.34
ET2	3.213	3.95	3.487	3.873	3.577	3.66	3.54	3.61
ET3	3.497	3.197	5.16	4.15	4.213	4.553	3.63	4.05
ET4	3.967	4.167	4.85	4.057	4.637	4.873	3.68	4.31
ET5	3.227	4.627	5.577	4.14	4.85	4.357	3.83	4.37
ET6	4.057	4.757	5.587	4.43	4.973	4.95	3.98	4.67
ET7	3.89	4.363	5.37	4.34	4.783	4.637	3.68	4.43
Mean	3.83	4.21	5.03	4.20	4.45	4.48	3.53	

	Isolates(A)	Media(B)	Inter(A×B)
SE(m)	0.009	0.009	0.006
CD (5%)	0.02	0.02	0.02

Dry weight of seven different isolates of *E. turcicum* in different liquid media

The dry weight of mycelial mat was recorded in different liquid media namely PDB, sabouraud's, oat meal agar, corn meal agar, czapek dox media, elliot's agar media and rose Bengal agar media and results were significant ($p = 0.01$). Among all liquid media oat meal broth was recorded maximum dry weight that was

0.48gm and rose Bengal broth recorded minimum dry weight that was 0.29gm, likewise isolate ET6 was recorded maximum dry weight that was 0.47gm and ET2 was minimum that was 0.32gm among all isolates (Table 6). As shown in the graph 4, isolate ET6 was shown maximum dry weight among all isolates and oat meal liquid media was recorded maximum dry weight among all different liquid media.

**Graph 4:** Dry weight of seven different isolates of *E. turcicum* in different liquid media**Table 6:** Dry weight of seven different isolates of *E. turcicum* in different liquid media

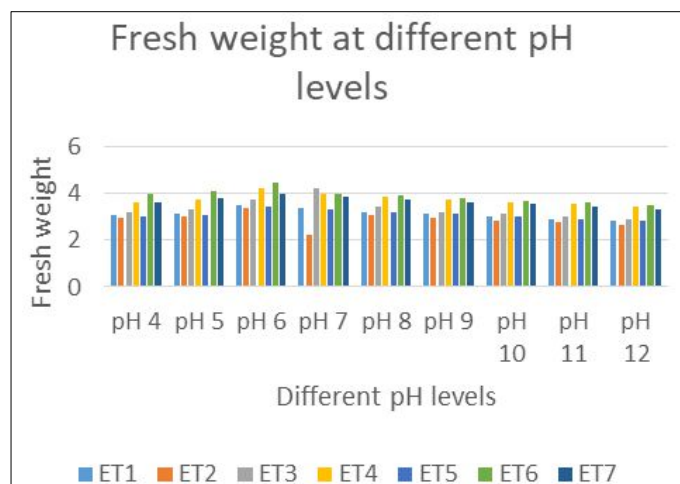
	PDB	Sabouraud's	Oat Meal	Corn Meal	Czapek's dox	Elliot's	Rose Bengal	Mean
ET1	0.33	0.42	0.50	0.41	0.43	0.47	0.32	0.41
ET2	0.31	0.37	0.31	0.31	0.39	0.37	0.23	0.32
ET3	0.32	0.43	0.51	0.42	0.43	0.47	0.37	0.42
ET4	0.38	0.43	0.49	0.43	0.44	0.48	0.38	0.43
ET5	0.37	0.48	0.52	0.44	0.45	0.47	0.39	0.44
ET6	0.40	0.50	0.54	0.43	0.57	0.50	0.40	0.47
ET7	0.40	0.47	0.51	0.42	0.48	0.47	0.38	0.45
Mean	0.34	0.44	0.48	0.40	0.45	0.46	0.29	

	Isolates(A)	Media(B)	Inter(A×B)
SE(m)	0.002	0.002	0.001
CD (5%)	0.005	0.005	0.003

Fresh weight of seven different isolates of *E. turcicum* at different pH levels

Fresh weight of seven different isolates was recorded at different pH levels namely pH4, pH5, pH6, pH7, pH8, pH9, pH10, pH11, pH12 and results were significant ($p = 0.01$). At pH6 maximum fresh weight was recorded that was 3.82gm,

at pH12 minimum fresh weight was recorded that was 3.08gm. For isolate ET6 maximum fresh weight was recorded that was 5.02gm and for ET2 it was minimum 3.7gm (Table 7). As shown in the Graph 6. Among all isolates ET6 was shown maximum fresh weight and among all different pH levels pH6 has shown maximum fresh weight.



Graph 5: Fresh weight of seven different isolates of *E. turcicum* at different pH levels

Table 7: Fresh weight (gm) of seven different isolates of *E. turcicum* at different pH levels

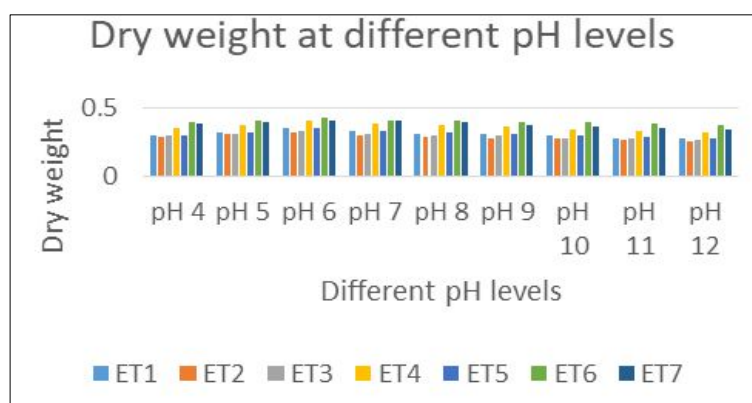
	pH4	pH5	pH6	pH7	pH8	pH9	pH10	pH11	pH12	MEAN
ET1	3.06	3.16	3.53	3.37	3.22	3.12	3.02	2.92	2.82	4.03
ET2	2.94	3.03	3.36	2.24	3.1	2.94	2.87	2.76	2.67	3.70
ET3	3.19	3.33	3.76	4.21	3.44	3.23	3.13	3.02	2.92	4.31
ET4	3.65	3.74	4.23	3.97	3.87	3.75	3.66	3.54	3.45	4.83
ET5	3.02	3.07	3.46	3.35	3.23	3.12	3.03	2.92	2.83	4.04
ET6	3.99	4.12	4.45	4.02	3.92	3.82	3.72	3.62	3.53	5.02
ET7	3.65	3.81	3.98	3.88	3.77	3.66	3.55	3.45	3.35	4.72
MEAN	3.35	3.46	3.82	3.57	3.50	3.37	3.28	3.17	3.08	

	Isolates(A)	pH Media(B)	Inter(A×B)
SE(m)	0.05	0.006	0.03
CD (5%)	0.1	0.2	0.09

Dry weight of seven different isolates of *E. turcicum* at different pH levels

Dry weight of seven different isolates was recorded at different pH levels namely pH4, pH5, pH6, pH7, pH8, pH9, pH10, pH11, pH12 and the results were significant ($p = 0.01$).

At pH6 maximum dry weight was recorded that was 0.37gm, at pH12 minimum dry weight was recorded that was 0.29gm. For isolate ET6 maximum dry weight was recorded that was 0.51gm and for ET2 it was minimum 0.36gm among all isolates (Table 8 & Graph 6.)



Graph 6: Dry weight of seven different isolates of *E. turcicum* at different pH levels

Table 8: Dry weight (gm) of mycelium at different pH levels

	pH4	pH5	pH6	pH7	pH8	pH9	pH10	pH11	pH12	MEAN
ET1	0.3	0.32	0.35	0.33	0.31	0.31	0.3	0.28	0.27	0.39
ET2	0.29	0.31	0.32	0.3	0.29	0.28	0.27	0.26	0.25	0.36
ET3	0.3	0.31	0.33	0.31	0.3	0.3	0.28	0.27	0.26	0.38
ET4	0.35	0.37	0.4	0.38	0.37	0.36	0.34	0.33	0.32	0.46
ET5	0.3	0.32	0.35	0.33	0.32	0.31	0.3	0.29	0.28	0.4
ET6	0.39	0.41	0.43	0.41	0.4	0.39	0.39	0.38	0.37	0.51
ET7	0.38	0.39	0.41	0.4	0.39	0.37	0.36	0.35	0.34	0.48
Mean	0.33	0.34	0.37	0.35	0.34	0.33	0.32	0.30	0.29	

	Isolates(A)	pH Media(B)	Inter(A×B)
SE (m)	0.0004	0.0004	0.0003
CD (5%)	0.001	0.001	0.0008

Conclusion

Study was conducted on seven isolates (ET1-ET7) of *Exserohilum turcicum* to assess their cultural and morphological characteristics. Colony color varied among isolates: ET1, ET2, and ET6 were greyish-black; ET4, ET5, and ET7 were black; while ET3 displayed a light greyish color. Despite the variation in colony color, all isolates exhibited black pigmentation. Sporulation levels also differed, with abundant sporulation observed in ET4 and ET6, good sporulation in ET1, ET3, and ET7, and poor sporulation in ET2 and ET5. Surface textures ranged from rough (ET1, ET2, ET4, ET6, and ET7) to fluffy (ET3 and ET5). The colony texture varied as well: ET1, ET2, ET3, and ET6 had cottony textures, ET5 and ET7 were adpressed, and ET3 additionally showed a velvety appearance. Colony margins showed differing branching patterns: ET6 and ET7 were highly branched, ET1, ET4, and ET5 were moderately branched, while ET2 and ET3 showed sparse branching. Radial growth of isolates was studied on seven solid media: Potato Dextrose Agar (PDA), Sabouraud's agar, Oatmeal agar, Corn Meal Agar (CMA), Czapek-Dox, Elliott's, and Rose Bengal Agar. Significant differences were observed ($p = 0.01$). Oatmeal agar supported the highest average radial growth (7.50 cm), while Rose Bengal recorded the least (4.18 cm). Among the isolates, ET6 exhibited the highest average growth (6.57 cm), and ET2 the lowest (3.42 cm). The isolates were also tested for growth at different pH levels (pH 4 to 12). The optimal pH was 6, where the highest average radial growth (6.76 cm) was recorded. Growth declined at extreme pH levels, with pH 12 showing the lowest (1.3 cm). Isolate ET4 showed the highest growth (7.94 cm), whereas ET2 showed the lowest (3.72 cm). Fresh and dry mycelial weights were measured in liquid media. Oatmeal broth yielded the highest fresh (5.03 g) and dry (0.48 g) weights, while Rose Bengal broth recorded the lowest (3.53 g and 0.29 g, respectively). ET6 was the most productive isolate in terms of biomass, with maximum fresh (4.67 g) and dry (0.47 g) weights. ET2 consistently showed the least biomass accumulation. At different pH levels, fresh and dry weights also varied significantly ($p = 0.01$). The optimal pH for fresh (3.82 g) and dry (0.37 g) weights was again pH 6, with ET6 producing the highest biomass (5.02 g fresh and 0.51 g dry), and ET2 the least (3.7 g fresh and 0.36 g dry). These findings highlight considerable variability in morphological and cultural traits among *E. turcicum* isolates. The data support the importance of field screening across early, medium, and QPM (Quality Protein Maize) genotypes for identifying resistant lines against Turcicum Leaf Blight (TLB). This foundational research offers valuable insights for future studies on host-pathogen interactions and disease resistance breeding.

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