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Isolation, Pathogenicity and Effect of Different Culture Media on *Exserohilum turcicum* Infecting Maize

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Abstract

Maize (*Zea mays* L.), the third most important cereal crop in India after rice and wheat, is highly vulnerable to foliar diseases that severely impact yield and quality. Among them, turcicum leaf blight (TLB), caused by *Exserohilum turcicum*, remains one of the most destructive, with yield losses reported up to 98 per cent under favourable conditions. The present study was conducted to isolate, identify and evaluate the pathogenicity of *E. turcicum*, as well as to assess its cultural behaviour on different solid media. Symptomatic maize leaves were collected and the pathogen was isolated using standard phytopathological techniques. Morphological studies revealed characteristic brown, septate hyphae and cylindrical conidia with a distinct hilum. Pathogenicity test on a susceptible maize cultivar (CM-202) confirmed the virulence of the isolate, producing typical cigar-shaped lesions under controlled conditions. For cultural characterization, the fungus was grown on ten different solid media. Potato dextrose agar (PDA) supported maximum mycelial growth while, oat meal agar (OMA) proved most conducive for sporulation. The results highlight the influence of nutrient composition and media type affectsfungal growth and reproduction, providing valuable insights for pathogen diagnosis, inoculum production and resistance screening in maize improvement programs.

Keywords : *Exserohilum turcicum*, Turcicum leaf blight, Maize, Pathogenicity, Media

Maize is the third most significant cereal crop in India after rice and wheat. Numerous bacterial, viral and fungal diseases can damage this crop. One of the significant foliar disease is turcicum leaf blight (TLB), which is caused by fungus teleomorphic fungi, *Setosphaeria turcica* (Luttrell) and its anamorphis *Exserohilum turcicum* (Passerini) Leonard and Suggs. The disease was initially described in Italy by Passerini, 1876 and in India by Butler, 1907. Andhra Pradesh, Karnataka, Bihar, Himachal Pradesh and Maharashtra are the states in India most affected by this disease in maize. Early disease epidemics causedblighted leaves to die prematurely and lose their nutritional value, as fodder (Sowmya *et al*., 2024). The majority of the composite and hybrid plants that are produced on a commercial scale are reported to be vulnerable to TLB. In cooler maize-growing locations, TLB was an endemic disease regarded to be crucial in terms of its geographic prevalence and ability to reduce production. When the leaves over the ear were impacted, even very slightly during the post-flowering period, losses from TLB are more severe (Shankara and Gowda, 2011). The most severe disease impacts

are produced by a warm, humid climate; late planting and maize grown from previous seasons (Blandino *et al*, 2012). Turcicum leaf blight is among the most devastating foliar diseases, causing serious reduction in grain yield of around 16-98 per cent (Reddy *et al*., 2014). In mid-elevation tropical zones with low temperatures, cloudy weather and excessive humidity during the maize growing season, TLB can be a major problem (Singh & Norong, 2005). If the symptoms appear prior to flowering, yield losses may exceed 50 per cent (Raymundo and Hooker, 1981).

Despite the increasing number of reports on *E. turcicum* infecting maize, there remain gaps in knowledge regarding how different culture media influence its growth and pathogenic behaviour under local conditions: media that are commonly used (such as PDA, V8 juice agar, malt extract agar etc.) may yield different growth rates, sporulation or differential virulence. In addition, the pathogenicity and aggressiveness of isolates may vary due to interaction of media, isolate, genotype and environmental conditions.

In this study, we aim to (i) isolate *E. turcicum* from infected maize plants in our region, (ii) confirm its pathogenicity on maize under controlled conditions and (iii) examine the effect of different culture media on its growth and development (colony morphology, sporulation, growth rate etc.). Understanding these aspects will help in disease diagnosis, phenotyping pathogen isolates and potentially in management strategies such as screening for resistant maize varieties or optimizing pathogen culturing techniques for research.

Material and Methods

Sample Collection, Fungal Isolation and Morphological Characterisation

A field survey was undertaken during the *Kharif* season of 2024-25 in Hassan district, Karnataka, India (12.99423°N, 76.11627°E). Maize leaves exhibiting characteristic blight symptomswere collected and brought to the Plant Pathology Laboratory, University of Agricultural Sciences, Bangalore (13.07918°N, 77.57101°E) for detailed investigation. Fungal isolation was carried out following standard phytopathological protocols. Leaf fragments (≈ 5 × 5 mm) encompassing diseased and adjoining healthy tissue were carefully excised with a sterile scalpel, surface-sterilized in 2 per cent sodium hypochlorite for 2 minutes, rinsed thrice in sterile distilled water and dried on sterile filter paper. The sterilized bits were aseptically placed at equidistant positions on Potato Dextrose Agar (PDA) plates and incubated at 27 ± 1 °C. Emerging colonies were purified on PDA supplemented with streptomycin and morphologically distinct isolates were retained for subsequent analyses. For cultural studies, 10-day-old colonies were observed to assess macroscopic features, while 3-week-old cultures were subjected to micro morphological examination using the slide culture method (Stevens, 1974). Microstructures were studied under a Leica DM750 compound microscope, enabling precise characterization of the fungal morphology.

Pathogenicity Assays

Pathogenicity of the isolated fungus was evaluated on the TLB-susceptible maize variety CM-202. Ten-day-old cultures were prepared and a conidial suspension (2 × 10v conidia/mL), quantified using a haemocytometer, was uniformly sprayed on healthy seedlings at the four-leaf stage. Control plants were mock-inoculated with sterile distilled water. To create a conducive microclimate, inoculated plants were covered with polythene sheets, ensuring high humidity for disease establishment. Plants were carefully monitored and observations on symptom initiation and disease progression were systematically recorded. The pathogen was subsequently re-isolated from infected tissues to fulfil Koch's postulates. For reliability and reproducibility, the assay was conducted with three independent replications.

Cultural Characterisation of *E. Turcicum* on Different Solid Media

The isolated fungus was cultured on ten distinct solid media-potato dextrose agar (PDA), corn meal agar (CMA), oat meal agar (OMA), sabouraud dextrose

agar (SDA), water agar (WA), potato dextrose carrot agar (PDCA), Richards agar (RA), Czapek's dox agar, malt extract agar (MEA) and V8 juice agar (VJA) to assess its cultural behaviour. Mycelial discs of 5 mm, cut from actively growing 7-day-old cultures, were inoculated at the centre of Petri plates containing the respective media and incubated at 27 ± 1 °C for one week. After seven days, observations were recorded on colony traits including texture, margin and pigmentation. Sporulation intensity was examined from three-week-old cultures using lactophenol-stained preparations under the microscope. Based on comparative assessment, sporulation on different media was categorized into classes ranging from absent to excellent (Table 1).

Table 1

Scoring and grading of sporulation

Score	Grade	Conidia/ microscopic field (100X)*
++++	Excellent	>75
+++	Good	51-75
++	Fair	26-50
+	Poor	1-25
-	No sporulation	-

Results and Discussion

Symptomatology, Pathogen Isolation and Morphological Characterization

Maize leaves infected by the pathogen exhibited elongated, elliptical, cigar-shaped lesions with a greyish-white centre surrounded by yellow halo, often tapering at both ends. These symptoms were most prominent during the late vegetative to early reproductive stages of crop growth. Under severe infection, individual lesions merged, producing extensive blighting of the foliage (Plate 1). Fungal isolation from symptomatic tissues consistently produced rapidly growing colonies on potato dextrose agar (PDA). The colonies initially appeared greyish-white but gradually turned dark brown to nearly black with age. Microscopic observations revealed brown, septate hyphae along with characteristic conidia that were straight to slightly curved, cylindrical in shape and bore a distinctly protruding hilum, an identifying hallmark of *E. turcicum* (Plate 2), visualized using a Leica DM750 compound microscope. Similar observations were also made by Anwer *et al*. (2022) and Patil *et al*. (2022).

Pathogenicity Test

The pathogenicity test on the susceptible maize variety, CM-202 revealed clear symptom development

Plate 1 : Infections showing cigar shaped lesions and leaf blight symptoms on maize. Field view (a), characteristic cigar shaped lesion on leaf blade, (b) multiple cigar shaped lesions on leaf blade (c)

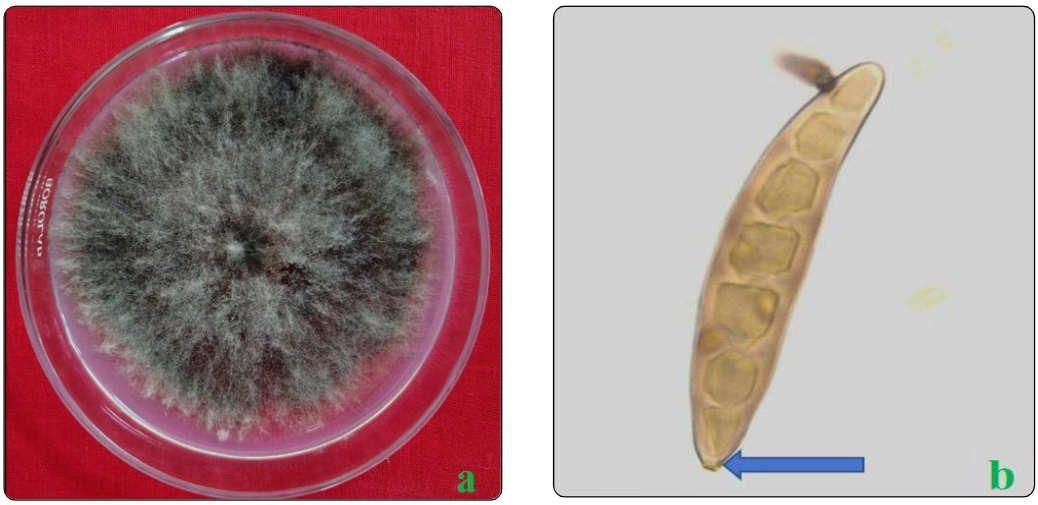

Plate 2 : Cultural and morphological characteristics of *E. turcicum*. (a) Colony of *E. turcicum* on potato dextrose agar medium and (b) conidial structure with arrow indicating hilum (blue arrow indicates hilum, a characteristic feature of *E. turcicum* conidia)

within 7-9 days after inoculation. The first symptom appeared as tiny, oval to round, yellowish spots that gradually expanded into elongated, elliptical to spindle-shaped necrotic lesions bordered by dark brown margins. With progression, these lesions matured into the characteristic form, displaying a greyish-white centre surrounded by yellow halo. The symptoms closely mirrored those observed under natural field conditions (Plate 1a). Similar observations were recorded by Abebe and Singburaudom (2006) and Anwer *et al*. (2022). In contrast, control plants treated with sterile water remained symptom-free, showing no spots or necrosis. The pathogen was successfully re-isolated from the artificially infected tissues and its cultural and morphological features matched those of the original

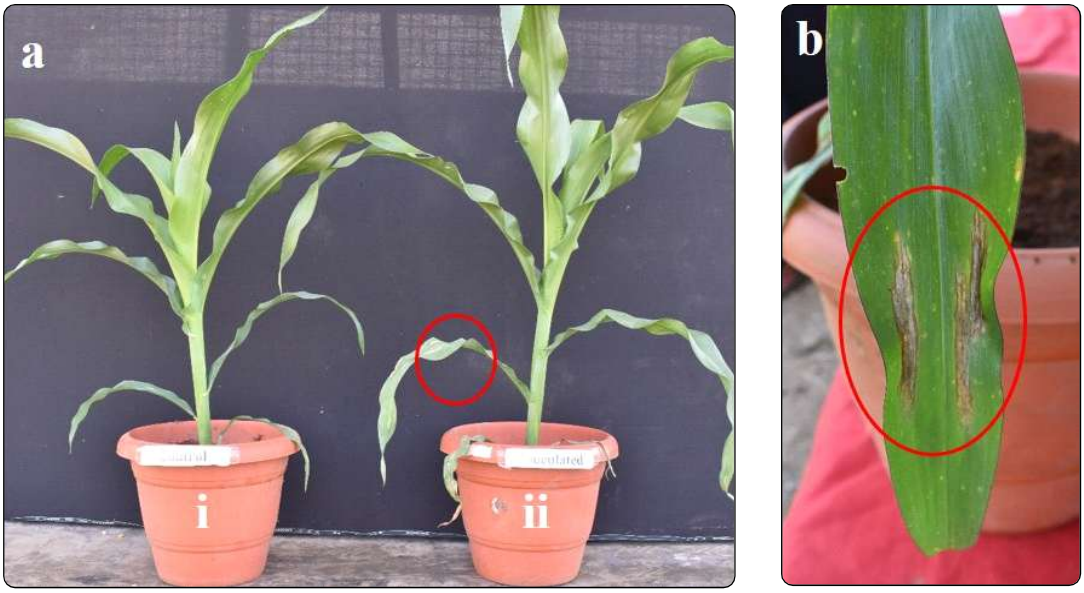

Plate 3 : Pathogenicity assay of *E. turcicum* on maize. (a) Control (i) and Inoculated (ii) plants, (b) cigar shaped lesions symptoms exhibited at 10 days after inoculation

field isolate, thereby confirming its identity and pathogenic role.

Cultural Characterisation of *E. turcicum* on Different Solid Media

The growth of *E. turcicum* was evaluated on ten different solid media and notable variations were observed in colony development. The highest radial extension was achieved on potato dextrose agar (90 mm), closely followed by corn meal agar (87.67 mm) and Richard's agar (85.67 mm), while the slowest growth occurred on Sabouraud's dextrose agar (32.33 mm). Colony texture ranged from leathery and smooth to fluffy and cottony; smooth colonies were common on PDA and water agar, whereas CDA and oat meal agar supported cottony growth. Prominent concentric rings were a characteristic feature on RA, CMA and malt extract agar, while PDCA and SDA showed no distinct ring formation. Hyphal margins varied between entire and undulating, with PDA, RA and MEA showing mostly entire edges. Colour expression ranged from light to dark grey on PDA, RA, CMA, WA, VJA and SDA, while OMA and MEA developed a greenish tint. Sporulation capacity was particularly high on OMA, whereas RA failed to support it. Altogether, these observations suggest that PDA provides the most vigorous growth, while OMA is highly favourable for prolific sporulation of *E. turcicum* (Table 2; Plate 4 and Fig. 1).

The present study demonstrates considerable cultural behaviour of *E. turcicum* across different solid media, highlighting the significant influence of nutrient composition and media type on fungal growth, development and sporulation by identifying PDA as the best media for pathogen growth, which is contrary to those of the isolates from cucumber (Dhara *et al*., 2020). Because of its simple sporulation, PDA allows for the best mycelial development of the fungus (Saha *et al*., 2008), but it includes too many nutrients, which leads to sporulation loss (UKNCC, 1998). High sporulation was found in OMA as this particular media is rich in nitrogen, carbon, proteins and nutrient (Murray *et al*., 2003). PDA and MEA also shows moderate to heavy sporulation as these fungal media also stimulate sporulation (MacFaddin, 1986).

The present investigation confirmed *E. turcicum* as the causal agent of TLB in maize through isolation, morphological characterization and pathogenicity assays. Typical cigar-shaped lesions and successful pathogen re-isolation validated its virulence on susceptible maize cultivars. Cultural studies demonstrated significant variation in growth and sporulation across different media, with PDA supporting maximum mycelial growth and OMA

TABLE 2

Effect of different media on mycelial growth and sporulation of *E. turcicum*

Different media	Radial growth	Colony texture	Concentric rings	Margin of the colony	Colony colour	Sporulation (10X)
PDA	90.00	Smooth	Less distinct rings	Entire	Dark grey	+++
RA	85.67	Leathery	Distinct rings	Entire	Dark grey	-
CMA	87.67	Leathery	Distinct rings	Entire	light grey	++
CDA	84.00	Cottony	Less distinct rings	Undulating	Olivaceous white	++
MEA	81.00	Fluffy	Distinct rings	Entire	Olivaceous green	+++
OMA	73.67	Cottony	Less distinct rings	Undulating	Olivaceous green	++++
PDCA	58.33	Cottony	No distinct rings	Undulating	Light grey	++
WA	48.67	Smooth	No distinct rings	Entire	Light grey	++
VJA	41.67	Fluffy	Less distinct rings	Entire	Light to dark grey	+
SDA	32.33	Leathery	No distinct rings	Undulating	Dark grey	++

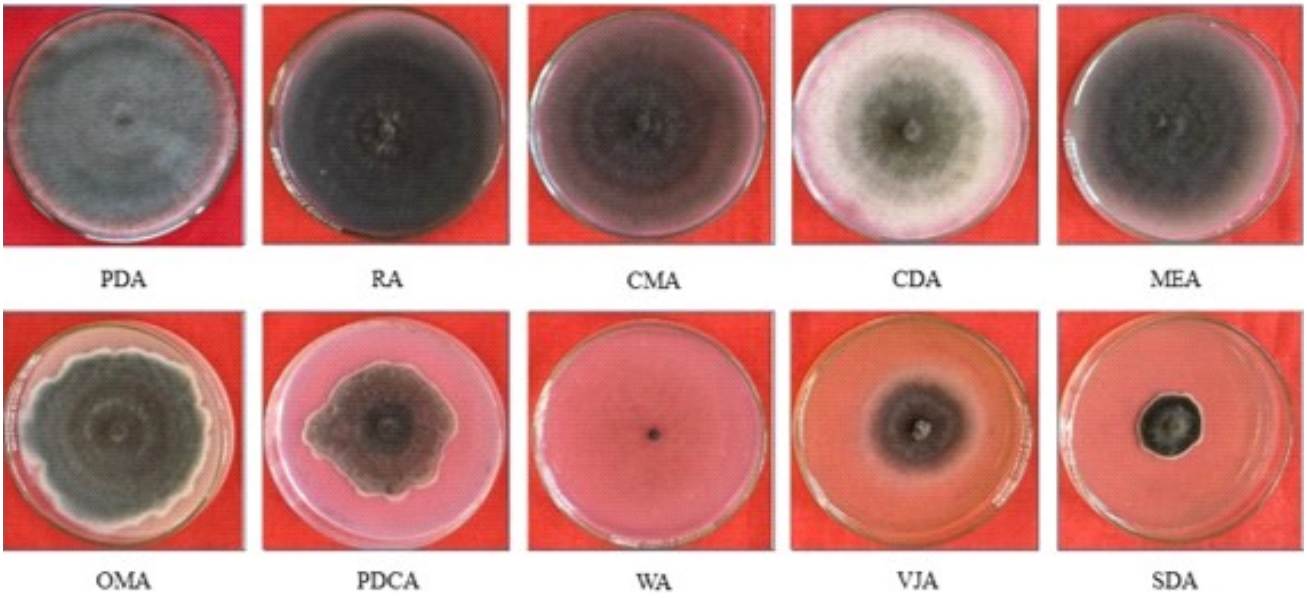

Plate 4 : Effect of different culture media on mycelial growth of *E. turcicum*

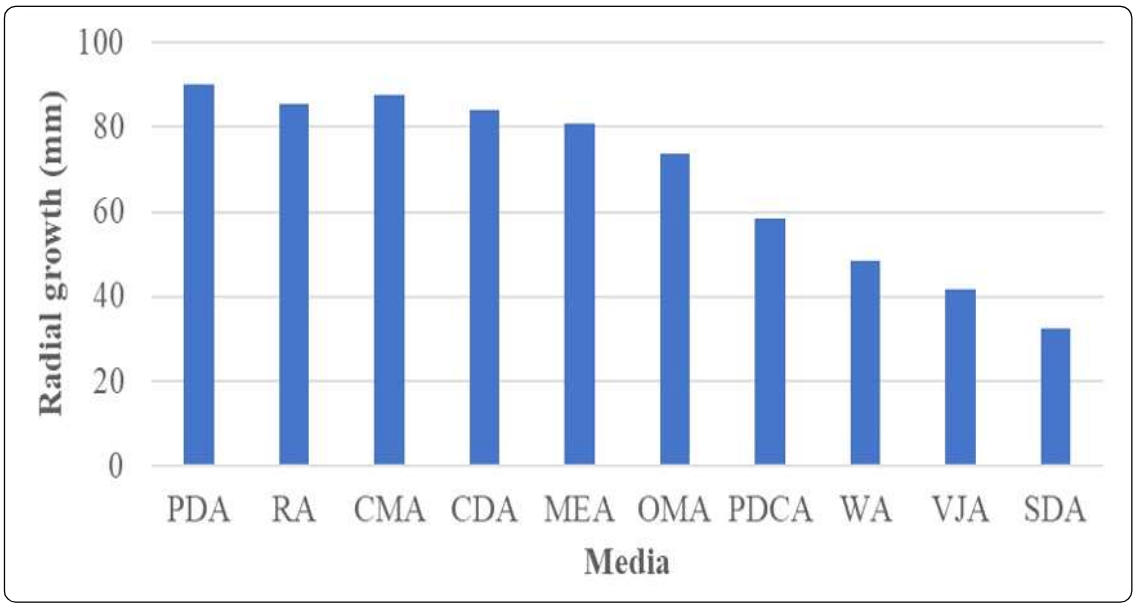

Fig. 1 : Effect of different culture media on mycelial growth of *E. turcicum*

proving most effective for sporulation. These findings highlight the importance of selecting suitable culture media for reliable pathogen maintenance and inoculum production. Overall, the study provides valuable insights for disease diagnosis, pathogen characterization and screening strategies in maize resistance breeding programmes.

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