

Journal of Biological Control, 39(2): 166-173, 2025, DOI: 10.18311/jbc/2025/48620

Research Article

In vitro evaluation of biocontrol agents and essential oils: Eco-friendly management of *Turcicum* leaf blight in maize

D. CHETHAN[1*], SRABANI DEBNATH[1,2], YERAKAM DURGA[1] and R. CHANDANA[3]

[1]*Department of Plant Pathology, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur – 741252, West Bengal, India*

[2]*AICRP on Maize, Bidhan Chandra Krishi Viswavidyalaya, Kalyani – 741235, West Bengal, India*

[3]*Department of Plant Pathology, University of Agricultural Sciences, Bangalore – 560065, Karnataka, India*

**Corresponding author E-mail:* chethangowda18sep@gmail.com

ABSTRACT: *Turcicum* Leaf Blight (TLB), caused by *Exserohilum turcicum*, is a devastating foliar disease of maize, leading to significant yield losses. This study aimed to isolate and characterise *E. turcicum* from infected maize fields in Kalyani, West Bengal, and evaluate the efficacy of biocontrol agents and essential oils against the pathogen. The pathogen was identified through morphological, cultural, and molecular analyses, including ITS sequencing and phylogenetic studies. *In vitro* assays revealed that *Trichoderma harzianum, T. asperellum, Bacillus subtilis*, and *Pseudomonas fluorescens* exhibited strong antagonistic activity, with the highest inhibition rates. Among essential oils, Tea Tree and Garlic oils exhibited complete inhibition of *E. turcicum*, while Turmeric and Tulsi oils showed initial effectiveness. These findings highlight the potential of biocontrol agents and essential oils as eco-friendly alternatives for managing TLB, offering sustainable strategies to reduce reliance on chemical fungicides and promote environmentally safe agricultural practices.

KEYWORDS: Biocontrol, eco-friendly, essential oils, *Exserohilum turcicum*

(Article chronicle: Received: 16-02-2025; Revised: 25-05-2025; Accepted: 27-05-2025)

INTRODUCTION

Maize (*Zea mays* L.) is a vital cereal crop globally, serving as a staple food, feed, and raw material for various industrial products. In India, it ranks third in cereal production after rice and wheat, and significantly contributes to food security and farmers' income, particularly in resource-poor areas. It is cultivated in diverse agro-climatic zones, with major maize-producing states including Karnataka, Madhya Pradesh, Maharashtra, Rajasthan, and Bihar. While India is a major maize producer. Despite its importance, maize productivity is often limited by various biotic and abiotic stresses. Among biotic factors, diseases pose a significant threat to maize production. TLB caused by the fungal pathogen *E. turcicum* (Pass.) Leonard and Suggs (Sethy *et al*., 2023) is a devastating foliar disease particularly prevalent in warm, humid regions that can lead to substantial yield losses (Raymundo & Hooker, 1981; Sibiya *et al*., 2013; Reddy *et al*., 2014; Hooda *et al*., 2017), particularly in warm and humid regions conducive to pathogen proliferation. The effective management of TLB is crucial for sustaining maize productivity. Current disease control practices heavily depend on synthetic fungicides, raising concerns about environmental pollution and the emergence of fungicide resistance in the pathogen. Hence, exploration of sustainable and eco-friendly alternatives for disease management is imperative. Biological control, involving the use of antagonistic microorganisms and natural products such as essential oils, offers a promising approach to suppressing plant diseases. Biocontrol agents, including antagonistic fungi and bacteria, provide a promising solution (Harlapur *et al*., 2007) and Essential Oils (EOs) derived from medicinal and aromatic plants have emerged as promising natural antifungal agents due to their broad-spectrum antimicrobial properties (Bhatt & Kumar, 2019). Hence, this study evaluates the efficacy of selected biocontrol agents and essential oils against *E. turcicum* under *in vitro* conditions, aiming to contribute to the development of sustainable disease management strategies for TLB disease in maize.

MATERIALS AND METHODS

Collection of the TLB disease sample

Maize leaves exhibiting characteristic TLB symptoms, such as necrotic lesions, were collected from farmers' fields in Kalyani during the grain-filling stage. Saples were stored in butter paper bags for transportation and subsequent pathogen isolation, which were properly labelled and refrigerated until further use, to prevent secondary contamination. The pathogen was isolated from the infected leaf tissues using the tissue isolation method and cultured on appropriate

media. The cultures were then stored at 4°C for further use in laboratory experiments.

Pathogen isolation and identification

Infected leaf samples were collected from the maize field and the infected leaf segments (5 mm²) were thoroughly washed in sterile distilled water. The samples were then surface-sterilised with 1% sodium hypochlorite (NaOCl) for 30 s, rinsed thrice with sterile distilled water, and subsequently dried on sterile filter paper. The sterilised leaf segments were plated onto a Potato Dextrose Agar (PDA) medium (Hi Media Pvt. Ltd., India), and the plates were sealed with Parafilm® (Anwer *et al*., 2022). The samples were incubated in a Biological Oxygen Demand (BOD) incubator at 25 ± 2°C for 24-48 h in the dark. The fungal colonies were monitored every 2-3 days until complete growth was observed. Once the colonies were fully developed, small segments of the fungal mycelium were subcultured to obtain pure isolates.

Cultural and morphological characterisation

Colony colour, pigmentation, sporulation and growth patterns on PDA were recorded after 12 days. The Munsell colour chart was used to determine the colour and pigmentation of culture (Reddy *et al*., 2014). The study focused on the morphological characteristics of conidia of *E. turcicum*. Specifically, the length, width and septation of the conidia were recorded. The data was recorded using a pre-calibrated Olympus CX21i LED compound microscope with an attached camera and Image View software.

Pathogenicity test

The pathogenicity of *E. turcicum* was assessed using Koch's postulates on one-month-old seedlings of the susceptible maize cv. Kaveri 50, grown in plastic pots containing sterilised soil under glasshouse conditions. A ten-day-old culture of *E. turcicum* was prepared, blended with sterilised distilled water, and filtered through cheesecloth. The spore concentration was adjusted to 4×10^5 spores per ml using a hemocytometer. Maize plants were inoculated by spraying the spore suspension onto the foliage using an atomiser, while a control group was sprayed with sterilised water instead of the spore suspension. To facilitate the pathogen's penetration into leaf tissue, the inoculated plants were covered with polythene bags (Abdulaziz *et al*., 2017). Disease symptom development was monitored regularly. Upon the appearance of symptoms, the infected leaf tissues were re-isolated, and the resulting fungus was compared with the original culture to confirm the identity of *E. turcicum*.

Molecular identification

DNA isolation

The genomic DNA was extracted using a modified CTAB method (Murray & Thompson, 1980). Potato Dextrose Broth (PDB) was used to culture *E. turcicum* for 10 days at 26±2°C. The collected fungus mat was ground into a fine powder with the aid of liquid nitrogen. The resulting extracts were then moved to sterile polypropylene tubes containing 15 mL of 2X CTAB extraction buffer, preheated to 65°C, and consisting of 2-3 per cent W/V CTAB, 1.4 M NaCl, 20 mM EDTA, 100 mM Tris-HCl, pH 8, and 0.17 per cent beta-mercaptoethanol. To purify the DNA, equal volumes of ethanol and chloroform-isoamyl alcohol were used. Following centrifugation at 10,000 rpm for 5 minutes, the supernatant was transferred to a new tube. Genomic DNA was precipitated by adding 0.6 ml of ice-chilled isopropanol, mixed by inversion, and then incubated on ice for 30 min. Then the samples were centrifuged at 10,000 rpm at 4°C for 10 min. After discarding the supernatant, the DNA was washed with 70 per cent ethanol, air-dried, and finally re-suspended in 100 µl of TE buffer (Singh *et al*., 2022; Anwer *et al*., 2022).

PCR amplification

The PCR amplification of DNA was effectively carried out using universal primers ITS-1 forward (5'-TCCGTAGGTGAACCTGCGG-3') and ITS-4 reverse (5'-TCCTCCGCTTATTGATATGC-3') (White *et al*., 1990; Anwer *et al*., 2022) on a thermal cycler. The PCR reaction had an overall volume of 30 µl, which included 2 µl of DNA template, 1.5 µl of forward and reverse primer, 10 µl of nuclease-free water and 15 µl of PCR master mix. The thermal cycler (Wee32, Himedia, model: LA1060) was programmed with an initial denaturation step at 96°C for 5 min, followed by 35 cycles of denaturation at 95°C for 60 s, annealing at 55°C for 60 s, extension at 72°C for 60 s, and final extension for 72°C for 5 min (White *et al*., 1990). The PCR products were then sent for sequencing using the Sanger sequencing method (Biokart India Pvt. Ltd., Bengaluru, Karnataka, India). The ITS sequence was aligned using MEGA11 software, and a BLASTn search was performed to compare it with sequences in the GenBank database (http://www.ncbi.nlm.nih.gov). The accession number was taken from NCBIGenBank, and a phylogenetic tree was constructed using MEGA11 software (Tamura *et al*., 2021).

In vitro* evaluation of biocontrol agents and essential oils against *E. turcicum

Evaluation of biocontrol agents against E. turcicum

Nine biocontrol agents (*T. harzianum* (Th-01, Th-02, Th-03 and Th-04), *T. viride* (Tv-01), *T. asperellum* (Ta-01), *B. subtilis* (Bs-01), *B. pumilus* (Bp-01), and *P. fluorescens* (Pf-01)) were previously isolated and maintained in the AICRP Maize laboratory, Kalyani Centre, West Bengal, India (Table 1). Four isolates of *T. harzianum* (Th-01, Th-02, Th-03 and Th-04), *T. viride* (Tv-01), *T. asperellum* (Ta-01), *B. subtilis* (Bs-01), *B. pumilus* (Bp-01), and *P. fluorescens*

(Pf-01) were evaluated for their antagonistic activity against *E. turcicum* using the dual culture technique. Each biocontrol agent and the pathogen were placed on opposite peripheral sides of PDA plates. Three replications were conducted for each treatment, with a control treatment in which the pathogen and biocontrol agents were maintained separately. The inoculated plates were incubated at 27±1°C for twelve days. The colony diameters of the pathogen were measured in two perpendicular directions, and the average values were recorded. The percentage inhibition of the pathogen's growth was calculated using the formula provided by Vincent (1947).

$$Percent\ inhibition = \frac{C-T}{C} \times 100$$

where,
C = colony diameter in control plates (mm)
T = colony diameter in treatment plates (mm)

In vitro evaluation of essential oils against E. turcicum

Eight commercially available essential oils (Naturalis Essence of Nature®) were tested for their antifungal efficacy against *E. turcicum* using the poison food technique. The pathogen, *E. turcicum*, was cultured on Potato Dextrose Agar (PDA) medium in Petri dishes and incubated for ten days at 25±1°C before experimentation. To prepare the essential oil suspensions, each essential oil was added to PDA to achieve a final concentration of 1 per cent. A total of 20 ml of the poisoned medium was then poured into sterilised Petri plates. A 0.5 cm diameter mycelial disc was excised from the periphery of the 10-day-old culture and placed at the centre of each Petri plate. Plates were incubated at 25±1°C for the duration of the experiment. A control group was maintained without the addition of any essential oils. All treatments, including the control, were conducted in triplicate. The diameter of the fungal colonies was measured in two perpendicular directions, and the average colony diameter was calculated. The percentage inhibition of fungal growth was determined using the formula provided by Vincent (1947).

Table 1. Details of biocontrol agents

S. No.	Biocontrol agents	NCBI Accession Number
1	*Trichoderma harzianum* (Th-01)	MZ562733
2	*Trichoderma harzianum* (Th-02)	PQ306375
3	*Trichoderma harzianum* (Th-03)	PQ557239
4	*Trichoderma harzianum* (Th-04)	PQ557290
5	*Trichoderma viride* (Tv-01)	PQ558472
6	*Trichoderma asperellum* (Ta-01)	PQ558609
7	*Pseudomonas fluorescens* (Pf-01)	PQ560712
8	*Bacillus subtilis* (Bs-01)	PQ560652
9	*Bacillus pumilus* (Bp-01)	PQ560752

RESULTS

Collection and isolation of the pathogen

The maize plants infested by TLB disease, was characterized by elongated, spindle-shaped necrotic lesions which measured up to 15 cm running parallel to the leaf margin (Figure 1). Small grey spots appeared on the lower leaves at all stages of the crop. They spread upwards as large, spindle-shaped spots by destroying the lower leaves and look like burnt areas due to the destruction of foliage.

Cultural and morphological characterisation

The isolated fungal culture, designated as Kalyani-TLB-01, was studied to examine its cultural characteristics. The colony exhibited a greyish–black colour with black pigmentation, regular margins and slightly fluffy texture (Figure 2a, b). The radial growth of the culture was profuse, reaching an average diameter of 84.30 mm on the 12th day post inoculation. Microscopic observations revealed that the conidia measured an average length of 89.02 µm and a width of 14.50 µm. The number of septa per conidium ranged from 5-10, and sporulation of 15x 10^4 spores/ml (Figure 2c, d).

Pathogenicity test

The pathogenicity of the Kalyani-TLB-01 isolate was confirmed using Koch's postulates on one-month-old maize seedlings (cv. Kaveri 50). Seedlings inoculated with a spore suspension (4 x 10^5 spores/mL) of the isolate developed characteristic symptoms of TLB within one week post-inoculation. These symptoms resembled those observed under natural field conditions 15 days after inoculation (Figure 3). The pathogen was successfully re-isolated from the infected leaf tissue, and its cultural and microscopic characteristics matched those of the original Kalyani-TLB-01 isolate, thereby fulfilling Koch's postulates and confirming its pathogenicity. In contrast, control plants, sprayed with sterile water, remained asymptomatic.



Figure 1. Symptomatology of TLB disease in maize.



Figure 2. Cultural and morphological characteristics of *E. turcicum* Kalyani-TLB-01 isolate. (a) Front view of Colony (b) Colony pigmentation (c) Conidia at 10X (d) Conidia at 40X.



Figure 3. Symptoms observed on the maize variety cv. Kaveri 50 during the pathogenicity test of Kalyani-TLB-01 isolate.

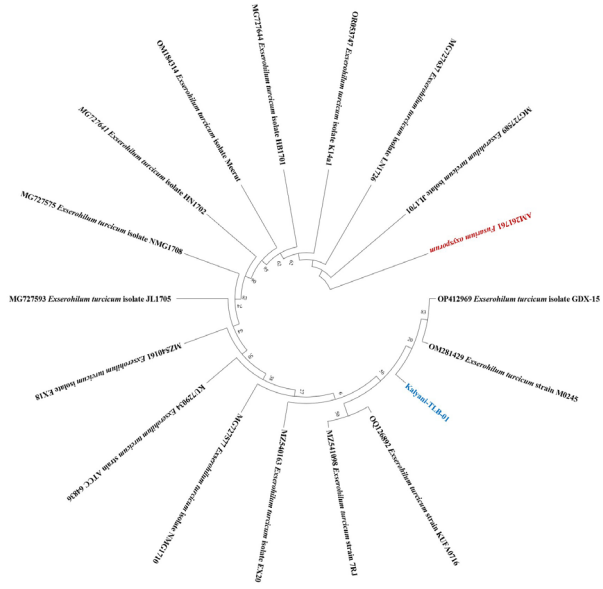


Figure 4. Phylogenetic tree of *E. turcicum* Kalyani-TLB-01 isolate.

Molecular identification and phylogenetic analysis

The genomic DNA from the Kalyani-TLB-01 isolate was extracted using the CTAB method. The ITS region was amplified by PCR using universal primers ITS 1 and 4 (White *et al*., 1990), resulting in approximately 600 bp. These PCR products were sequenced, and the resulting sequence was analysed using NCBI's (National Centre for Bioinformatics) Nucleotide BLAST tool (www.blast.ncbi.nlm.nih.gov/blast). Alignment with known sequences revealed that the Kalyani-TLB-01 isolate is most similar to *E. turcicum,* and sequences were deposited in the NCBI GenBank with obtained accession number PV123152.

A phylogenetic analysis was then performed to confirm the identity of Kalyani-TLB-01 and elucidate its evolutionary relationships. The ITS sequence from the isolate was aligned with the ITS sequences of *E. turcicum* retrieved from the NCBI GenBank database, with *Fusarium oxysporum* (Accession AM261761) used as the outgroup. The phylogenetic tree was constructed using the Neighbour-Joining (NJ) approach implemented in MEGA version 11. The bootstrap analysis was performed with 1,000 replicates to assess the robustness of the branching patterns. The phylogenetic tree (Figure 4) revealed that the isolate Kalyani-TLB-01 clusters strongly with other *E. turcicum* strains, confirming its identity.

In vitro evaluation of biocontrol agents against E. turcicum

A dual culture assay evaluated the antagonistic potential of various fungal and bacterial biocontrol agents against *E. turcicum (Figure 5;* Supplementary Table 1). Among the fungal antagonists, *T. harzianum* isolates Th-04 and Th-01, along with *Trichoderma asperellum* Ta-01, demonstrated the strongest inhibitory effects, achieving 78.46%, 77.77% and 78.46% inhibition, respectively. *T. harzianum* Th-02 also exhibited strong inhibition at 77.1%. While still effective, *T. harzianum* Th-03 (62.56%) and *Trichoderma viride* Tv-01 (61.43%) showed comparatively lower levels of inhibition (Figure 6). These results highlight the potential of *Trichoderma* species, particularly *T. harzianum* and *T. asperellum*, as effective biocontrol agents against *E. turcicum*. Within the bacterial group, *B. subtilis* Bs-01 proved to be the most potent antagonist, exhibiting 78.52% inhibition. *P. fluorescens* Pf-01 also demonstrated significant antagonistic activity, resulting in 72.59% inhibition *(Figure 7)*. However, *Bacillus pumilus* Bp-01 was ineffective, showing no inhibition of *E. turcicum* growth. This suggests that *B. subtilis* and *P.*

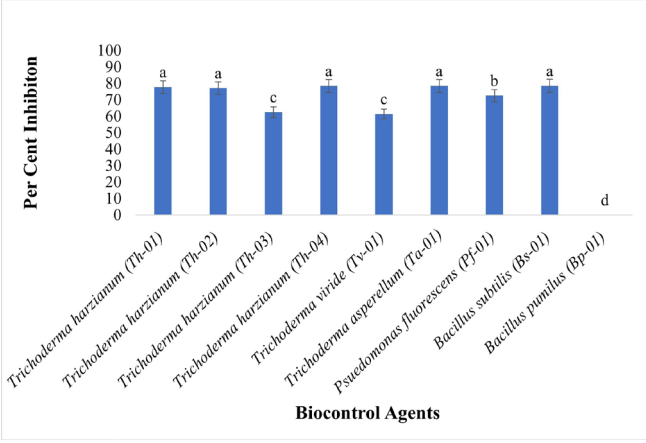


Figure 5. Per cent inhibition of *E. turcicum* Kalyani-TLB-01 isolate against biocontrol agents.

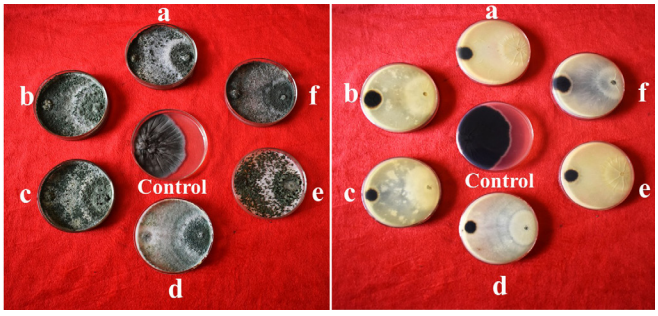


Figure 6. *In vitro* evaluation of fungal biocontrol agents against *E. turcicum* Kalyani-TLB-01 isolate.

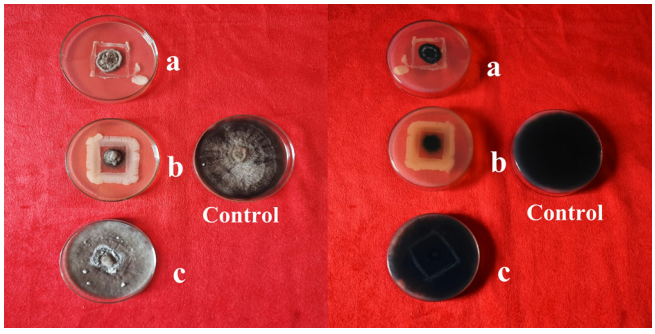


Figure 7. *In vitro* evaluation of bacterial biocontrol agents against *E. turcicum* Kalyani-TLB-01 isolate.

fluorescens hold promise as bacterial biocontrol agents for managing *E. turcicum*. Both fungal and bacterial biocontrol agents demonstrated strong antagonistic activity against *E. turcicum,* highlighting their efficacy as biological control strategies and could be integrated into disease management strategies to enhance their effectiveness against *E. turcicum*.

In vitro evaluation of essential oils against E. turcicum

The antifungal activity of various essential oils at 1% concentration against *E. turcicum* was assessed using the poisoned food technique (Figure 8), with daily observations

Supplementary Table 1. Per cent inhibition of *E. turcicum* against biocontrol agents

S. No.	Biocontrol agents	Per cent inhibition
1	*Trichoderma harzianum* (Th-01)	77.77 (8.87)[a]
2	*Trichoderma harzianum* (Th-02)	77.10 (8.83)[a]
3	*Trichoderma harzianum* (Th-03)	62.56 (7.97)[c]
4	*Trichoderma harzianum* (Th-04)	78.46 (8.91)[a]
5	*Trichoderma viride* (Tv-01)	61.43 (7.90)[c]
6	*Trichoderma asperellum* (Ta-01)	78.46 (8.91)[a]
7	*Pseudomonas fluorescens* (Pf-01)	72.59 (8.57)[b]
8	*Bacillus subtilis* (Bs-01)	78.52 (8.91)[a]
9	*Bacillus pumilus* (Bp-01)	0.00 (1)[d]
SE(m)		0.04
SE(d)		0.057
C.V. (%)		0.891

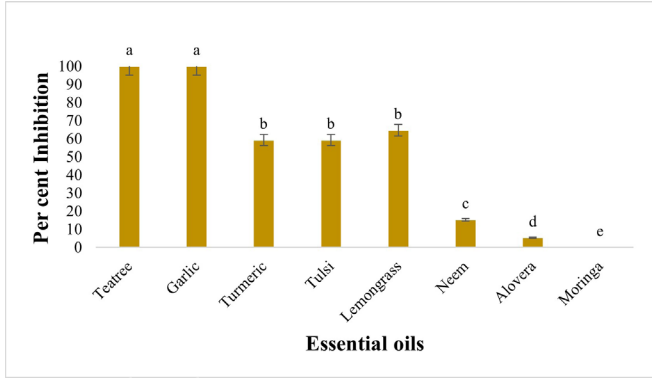


Figure 8. Per cent inhibition of *E. turcicum* Kalyani-TLB-01 isolate against essential oils.

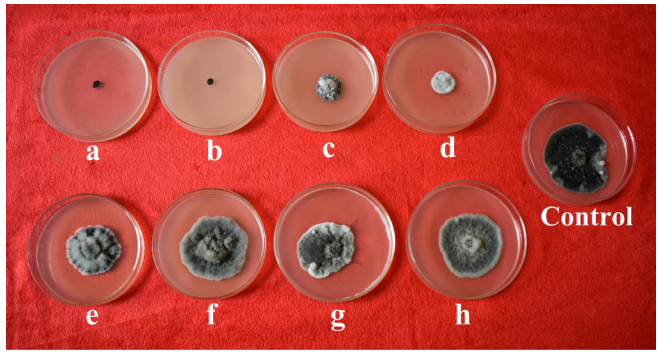


Figure 9. *In vitro* evaluation of essential oils against *E. turcicum* Kalyani-TLB-01 isolate.

recorded over ten days. Tea tree and Garlic oils achieved complete inhibition (100%) throughout the entire study. Turmeric and Tulsi oil maintained 100% inhibition for the first five days, gradually declining to 58.94% and 59.34%, respectively, by the tenth day. Lemongrass oil showed 100% inhibition on the first two days, dropping to 49.86% on the third day and fluctuating between 58.11 and 68.51%

Supplementary Table 2. Per cent inhibition of *E. turcicum* against EO

S. No.	Essential oils	Per cent inhibition of *Exserohilum turcicum*									
		1st day	2nd day	3rd day	4th day	5th day	6th day	7th day	8th day	9th day	10th day
1	Teatree	100 (10.05)^{a}	100 (10.05)^{a}	100 (10.05)^{a}	100 (10.05)^{a}	100 (10.05)^{a}	100 (10.05)^{a}	100 (10.05)^{a}	100 (10.05)^{a}	100 (10.05)^{a}	100 (10.05)^{a}
2	Garlic	100 (10.05)^{a}	100 (10.05)^{a}	100 (10.05)^{a}	100 (10.05)^{a}	100 (10.05)^{a}	100 (10.05)^{a}	100 (10.05)^{a}	100 (10.05)^{a}	100 (10.05)^{a}	100 (10.05)^{a}
3	Turmeric	100 (10.05)^{a}	100 (10.05)^{a}	100 (10.05)^{a}	100 (10.05)^{a}	100 (10.05)^{a}	69.79 (8.41)^{bc}	70.89 (8.47)^{b}	65.35 (8.14)^{c}	58.93 (7.741)^{c}	59.30 (7.76)^{b}
4	Tulsi	100 (10.05)^{a}	100 (10.05)^{a}	100 (10.05)^{a}	100 (10.05)^{a}	100 (10.05)^{a}	76.04 (8.77)^{b}	77.27 (8.84)^{b}	77.16 (8.84)^{b}	60.45 (7.83)^{bc}	59.33 (7.76)^{b}
5	Lemongrass	100 (10.05)^{a}	100 (10.05)^{a}	49.86 (7.12)^{b}	59.95 (7.806)^{b}	58.11 (7.68)^{b}	64.58 (8.09)^{c}	67.26 (8.26)^{b}	68.51 (8.33)^{c}	67.15 (8.25)^{b}	64.65 (8.10)^{b}
6	Neem	16.12 (4.03)^{b}	17.42 (4.21)^{b}	17.22 (4.22)^{c}	9.23 (3.199)^{c}	12.16 (3.59)^{c}	12.5 (3.65)^{d}	17.26 (4.27)^{c}	19.69 (4.54)^{d}	9.69 (3.26)^{d}	15.29 (4.02)^{c}
7	Aloe vera	16.43 (4.14)^{b}	8.58 (2.79)^{b}	0.00 (1)^{e}	0.00 (1)^{e}	0.00 (1)^{e}	0.00 (1)^{e}	0.00 (1)^{e}	4.70 (2.35)^{e}	9.69 (2.48)^{e}	5.29 (2.45)^{d}
8	Moringa	6.36 (2.49)^{b}	6.06 (2.45)^{b}	6.52 (2.74)^{d}	4.617 (2.371)^{d}	4.05 (2.24)^{d}	1.04 (1.34)^{e}	2.70 (1.81)^{d}	0.00 (1)^{f}	0.00 (1)^{f}	0.00 (1)^{e}
SE(m)		0.369	0.465	0.174	0.044	0.119	0.155	0.164	0.109	0.071	0.152
SE(d)		0.522	0.658	0.245	0.063	0.168	0.219	0.231	0.154	0.100	0.214
C.V. (%)		8.388	10.800	4.350	1.128	3.001	4.167	4.295	2.836	1.934	4.103

thereafter. Neem oil displayed low antifungal activity, inhibiting growth by 9.24 to 19.69%. Aloe vera oil showed minimal inhibition, peaking at 16.43% on the first day and declining to 5.29% by day ten. Moringa oil exhibited the weakest antifungal activity, with inhibition ranging from 0 to 6.53% (Supplementary Table 2). Tea tree and Garlic oils completely suppressed *E. turcicum* growth across ten days. Turmeric and Tulsi oils were effective initially, but lost potency over time. Lemongrass and Neem oils exhibited low antifungal activity. Aloe vera and Moringa oils had negligible inhibitory impact (Figure 9).

DISCUSSION

The isolation and characterisation of *E. turcicum* from maize fields in Kalyani, West Bengal, confirm its role as the causal agent of TLB disease. The symptoms observed, including spindle-shaped necrotic lesions and extensive foliar destruction, align with previous reports (Hooda *et al*., 2017). The successful isolation of the pathogen on potato dextrose agar, the growth of fungal colonies on PDA, characterised by a greyish-black colony colour, black pigmentation, and a slightly fluffy texture, is consistent with the descriptions by (Reddy *et al*., 2014; Rani *et al*., 2016; Abdulaziz *et al*., 2017; Patil *et al*., 2022). Microscopic characteristics, including conidial length (89.02 µm), conidial width (14.50 µm), and septation (5-10 septa per conidium), are within the reported range for *E. turcicum*, confirming its identity (Harlapur *et al*., 2007; Reddy *et al*., 2014; Rani *et al*., 2016). The pathogenicity test performed on maize seedlings confirmed the virulence of *E. turcicum*, fulfilling Koch's postulates. The development of characteristic TLB symptoms within a week of inoculation with a spore suspension (4×10^5 spores/ml) is indicative of the pathogen's high infectivity. Re-isolation of the pathogen and confirmation of identical morphological characteristics eliminate the possibility of secondary infections. To confirm the identification at the molecular level, ITS sequencing and phylogenetic analysis were conducted. The ITS region, a widely used marker for fungal identification, produced a PCR amplicon of 600bp (Zeigler *et al*., 1995; Abdulaziz *et al*., 2017; Anwer *et al*., 2022). NCBI-BLASTn analysis revealed a 96.14% homology with *E. turcicum*, further corroborating the identity of the isolate. The ITS sequence was deposited in GenBank under the accession number PV123152. A maximum-likelihood phylogenetic tree, constructed using MEGA 11, placed the isolate within the *E. turcicum* clade, confirming the pathogen's identity. The molecular and phylogenetic evidence strongly supports the identification of Kalyani-TLB-01 as *E. turcicum*,

The *in vitro* evaluation of biocontrol agents and essential oils against *E. turcicum Kalyani-TLB-01* isolate reveals promising alternatives for managing this pathogen. Among fungal antagonists, *T. harzianum* and *T. asperellum* exhibited strong inhibitory effects, aligning with previous studies that highlight the mycoparasitic and enzyme-producing capabilities of *Trichoderma* species in suppressing phytopathogens (Ray *et al*., 1990; Biswas *et al*., 2000; Viviana *et al*., 2006; Sartori *et al*., 2017). The bacterial

antagonist *B. subtilis* showed the highest inhibition, followed by *P. fluorescens,* corroborating reports on the biocontrol potential of *Bacillus* and *Pseudomonas* through antimicrobial metabolite production and competitive exclusion (Harlapur *et al*., 2007). The antifungal activity of essential oils demonstrated that Tea Tree and Garlic oils provided complete inhibition throughout consistent with their known antifungal compounds, such as terpinen-4-ol in Tea Tree oil and allicin in Garlic oil. Turmeric and Tulsi oils initially inhibited *E. turcicum* but gradually lost efficacy. Lemongrass oil, Neem oil, Aloe vera and Moringa oils exhibited the lowest inhibition, suggesting their bioactive compounds may not effectively target *E. turcicum (*Kishore *et al*., 2007; Bhatt & Kumar, 2019). The combined use of biocontrol agents and essential oils represents a holistic approach to managing TLB in maize. While biocontrol agents provide long-term disease suppression through colonisation and competition, essential oils offer rapid and direct antifungal effects. The integration of these biological control methods can potentially reduce reliance on chemical fungicides, mitigate the risk of pathogen resistance and promote sustainable maize cultivation.

CONCLUSION

This study revealed *E. turcicum* as the causal agent of TLB of maize in Kalyani, West Bengal, confirming its identity through morphological, cultural, and molecular analyses, including ITS sequencing and phylogenetic analysis. Furthermore, *in vitro* evaluations identified promising biocontrol agents, particularly *T. harzianum, T. asperellum, B. subtilis* and *P. fluorescens*, as well as effective essential oils, notably Tea Tree and Garlic, for potential integration into sustainable disease management strategies against this important maize pathogen. These findings contribute valuable information for developing an integrated disease management strategy against *E. turcicum* and promoting sustainable and environmentally friendly agricultural practices. Further, field trials are needed to validate the *in vitro* efficacy of promising biocontrol agents and essential oils against *E. turcicum* under natural conditions.

ACKNOWLEDGEMENTS

All the authors are thankful to the AICRP on Maize-Kalyani Centre, DR Building, Bidhan Chandra Krishi Viswavidyalaya, Kalyani, West Bengal and Department of Plant Pathology, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, West Bengal, for providing an infrastructure facility.

REFERENCES

Abdulaziz, B. K., Kamaruzaman, S., Khairulmazmi, A., Zulkifli, A. S., Mohd, S., and Norzihan, A. 2017. Characterisation and pathological variability of *Exserohilum turcicum* responsible for causing Northern Corn Leaf Blight (NCLB) disease in Malaysia. *Malays J Microbiol*, **13**(1): 41-49.

Anwer, M. A., Niwas, R., Ranjan, T., Mandal, S. S., Ansar, M., Srivastava, J. N., …, Bharti, A. 2022. Molecular and morphological characterisation of *Exserohilum turcicum* (Passerini) Leonard and Suggs causing Northern Corn Leaf Blight of maize in Bihar. *Bioeng*, **9**(8): Article 403. https://doi.org/10.3390/bioengineering9080403 PMid:36004928 PMCid:PMC9405478

Bhatt, B., and Kumar, P. 2019. Bioefficacy of fungicides, plant extracts and essential oils against *Exserohilum turcicum* (Pass.) Leonard and Suggs causing northern leaf blight of maize. *J Appl Nat Sci*, **11**(2): 257-262. https://doi.org/10.31018/jans.v11i2.2030

Biswas, S. K., Srivastava, K. D., Aggarwal, R., Dureja, P., and Singh, D. V. 2000. Antagonism of *Chaetomium globosum* to *Drechslera sorokiniana*, the spot blotch pathogen of wheat. *Indian Phytopathol*, **53**(4): 436-440.

Harlapur, S. I., Kulkarni, M. S., Yeshoda, H., and Srikant, K. 2007. Variability in *Exserohilum turcicum* (Pass.) Leonard and Suggs., causal agent of turcicum leaf blight of maize. *Karnataka J Agric Sci*, **20**(3): 665-666.

Hooda, K. S., Khokhar, M. K., Shekhar, M., Karjagi, C. G., Kumar, B., Mallikarjuna, N., …, Yadav, O. P. 2017. Turcicum leaf blight – Sustainable management of a re-emerging maize disease. *J Plant Dis Prot*, **124**(2): 101-113. https://doi.org/10.1007/s41348-016-0054-8

Kishore, G. K., Pande, S., and Harish, S. 2007. Evaluation of essential oils and their components for broad-spectrum antifungal activity and control of late leaf spot and crown rot diseases in peanut. *Plant Dis*, **91**(3): 375-379. https://doi.org/10.1094/PDIS-91-4-0375 PMid:30781177

Murray, M. G., and Thompson, W. F. 1980. Rapid isolation of high molecular weight plant DNA. *Nucleic Acids Res*, **8**(19): 4321-4326. https://doi.org/10.1093/nar/8.19.4321 PMid:7433111 PMCid:PMC324241

Patil, L. P., Jagtap, G. P., Hingole, D. G., Pawar, S. Y., and Gaikwad, G. B. 2022. Studies on morphological, cultural, physiological and pathological variability in isolates of *Exserohilum turcicum*, incitant of northern leaf blight of maize. *J Pharm Innov*, **11**(12): 702-708.

Rani, S., Gupta, S., and Singh, S. K. 2016. Morpho-cultural variability in *Exserohilum turcicum* isolates of Jammu. *Indian Phytopathol*, **69**(4s): 138-143.

Ray, S., Ghosh, M., and Mukharjee, N. 1990. Fluorescent *Pseudomonas* for plant disease control. *J Mycopathol Res*, **20**(2): 135-140.

Raymundo, A. D., and Hooker, A. L. 1981. Measuring the relationship between northern corn leaf blight and yield losses. *Plant Dis*, **65**(4): 325-327. https://doi.org/10.1094/PD-65-325

Reddy, T. R., Reddy, P. N., Reddy, R. R., and Reddy, S. S. 2014. Cultural and morphological variability among *Exserohilum turcicum* isolates causing turcicum leaf blight of maize. *Environ. Ecol*, **32**(1): 16-21.

Sartori, M., Nesci, A., Garcia, J., Passone, M. A., Montemarani, A., and Etcheverry, M. 2017. Efficacy of epiphytic bacteria to prevent northern leaf blight caused by *Exserohilum turcicum* in maize. *Rev Argent Microbiol*, **49**(1): 75-82. https://doi.org/10.1016/j.ram.2016.09.008 PMid:28188093

Sethy, S. K., Nongmaithem, N., Sinha, B., Singh, K. I., Singh, N. O., and Priyadarshini, M. 2023. Morphological and cultural variability among *Exserohilum turcicum* isolates causing turcicum leaf blight disease of maize in Manipur. *Int J Bio-Resour Stress Manag*, **14**(7): 1052-1060. https://doi.org/10.23910/1.2023.3495

Sibiya, J., Tongoona, P., Derera, J., and Makanda, I. 2013. Smallholder farmers' perceptions of maize diseases, pests, and other production constraints, their implications for maize breeding and evaluation of local maize cultivars in KwaZulu-Natal, South Africa. *Afr J Agric Res*, **8**(17): 1790-1798. https://doi.org/10.5897/AJAR12.1906

Singh, P., Huang, S. Y., Hernandez, A. G., Adhikari, P., Jamann, T. M., and Mideros, S. X. 2022. Genomic regions associated with virulence in *Setosphaeria turcica* identified by linkage mapping in a biparental population. *Fungal Genet Biol*, **159**: Article 103655. https://doi.org/10.1016/j.fgb.2021.103655 PMid:34954385

Tamura, K., Stecher, G., and Kumar, S. 2021. MEGA11: Molecular evolutionary genetics analysis version 11. *Mol Biol Evol*, **38**(7): 3022-3027. https://doi.org/10.1093/molbev/msab120 PMid:33892491 PMCid:PMC8233496

Vincent, J. M. 1947. Distortion of fungal hyphae in the presence of certain inhibitors. *Nature*, **159**(4036): 239-241. https://doi.org/10.1038/159850b0 PMid:20343980

Viviana, V., Varas, T. A. R., Freeman, N. C., Gudmestad, N. C., and Secor, G. A. 2006. Mycoparasitism of *Helminthosporium solani* by *Acremonium strictum*. *Indian Phytopathol*, **59**(1): 1331-1337. https://doi.org/10.1094/PHYTO-97-10-1331 PMid:18943692

White, T. J., Bruns, T., Lee, S.-J., and Taylor, J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In M. A. Innis, D. H. Gelfand, J. J. Sninsky, & T. J. White (Eds.), *PCR protocols: A guide to methods and applications* (pp. 315-322). Academic Press. https://doi.org/10.1016/B978-0-12-372180-8.50042-1 PMid:1696192

Zeigler, R. S., Cuoc, L. X., Scott, R. P., Bernado, M. A., Chen, D. H., Valent, B., and Nelson, R. J. 1995. The relationship between lineage and virulence in *Pyricularia grisea* in the Philippines. *Phytopathology*, **85**(4): 443-451. https://doi.org/10.1094/Phyto-85-443