Research Article

Morpho-Molecular Identification, Virulence and *In Vitro* Sporulation of Fungi Associated with Maize Foliar Diseases in Egypt

Eihab Mohamed Taha*, Elhamy M. El-Assiuty, Zeinab M. Fahmy and Doaa A. Kafsheer

Maize, Sugar and Forage Crops Diseases Department, Plant Pathology Research Institute, Agricultural Research Center, Giza, Egypt.

Abstract | Maize (*Zea mays* L.) is an important crop in Egypt. Foliar diseases including northern maize leaf blight (NCLB), southern corn leaf blight (SCLB), and Curvularia leaf spot (CLS) pose considerable economic threats to its yield. This study aimed to identify the primary fungal pathogens responsible for foliar blight and spot symptoms on maize in Egypt. Their pathogenic variability on both maize and sorghum was assessed, and optimal media for *in vitro* sporulation were determined. A survey in high-incidence areas in Egypt led to the collection of 15 symptomatic samples, from which seven single-spore fungal isolates were obtained. Pathogenicity experiments on maize showed varying virulences. Isolates Eg_57 and Eg_60 had the highest disease index, recording 88.75% and 83.75% respectively. Furthermore, only isolates Eg_52 and Eg_60 demonstrated cross-pathogenicity on sorghum. Morphological features of conidia and colony traits, supported by molecular characterization (ITS sequencing), identified the isolates as five isolates of *Bipolaris maydis* (Eg_57, Eg_59, Eg_60, Eg_73, and Eg_88), one isolate of *Exserohilum turcicum* (Eg_52), and single isolate of *Curvularia lunata* (Eg_23). Phylogenetic analysis validated their identities, with strong bootstrap support (100%). A considerable interaction between isolate and media was observed for sporulation. Of the seven media tested, potato dextrose agar with maize leaf pieces (MLP) produced the most count of spores across all tested isolates, averaging 48.3×10^4 spores/mL. These results suggested *B. maydis* as the most prevalent pathogen, and MLP was the most effective medium for conidia production. This research provides a basis for future epidemiological investigations and resistance-breeding programs.

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***Correspondence** | Eihab Mohamed Taha, Maize, sugar and Forage Crops Diseases Department, Plant Pathology Research Institute, Agricultural Research Center, Giza, Egypt; **Email:** eihab111@gmail.com
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Introduction

Maize (*Zea mays* L.) is the most versatile and productive grain crop, cultivated for food, feed, and oil and starch production (Sukop *et al.*, 2026). In Egypt, maize is the most widely grown cereal crop, with a cultivation area expanding from 1.78 million acres in 2007 to 2.25 million acres by 2021. During the same period, production increased from 6.14 million tons to 7.45 million tons (Adly Abd Elazim *et al.*, 2024).

Maize is threatened by pathogenic fungi, bacteria, and viruses that considerably reduce yields, resulting in annual crop losses of 23% (Benjamin *et al*., 2024). Northern corn leaf blight (NCLB), southern corn leaf blight (SCLB) and Curvularia leaf spot (CLS) were the three foliar fungal diseases approximately responsible for over 4% of these yield losses (Nsibo *et al*., 2021; Ranganatha *et al*., 2021; Berger, 2024). NCLB is caused by *Exserohilum turcicum* (Pass.) K.J. Leonard and E.G. Suggs (Synonyms: *Helminthosporium turcicum* teleomorph: *Setosphaeria turcica* (Luttell) K.J. Leonard and E.G. Suggs) (Leonard and Suggs, 1974). Worldwide, NCLB has been regarded as a serious foliar maize disease (Li *et al*., 2025). In addition, some isolates of *E. turcicum* have the ability to infect both maize and sorghum (Cui *et al*., 2024). The symptoms of NCLB are manifested in susceptible plants as elliptical, grayish-green, and later on as tan lesions on the lower leaves due to fungal sporulation. The lesions vary in size as they develop but are usually 2-30 cm long and 1-1.25 cm wide (Vieira *et al*., 2014). This disease can reduce yields by 30–50% in susceptible hybrids before the appearance of the tassels. However, when the disease is mild or develops after the appearance of the tassels, its impact on yield is usually minimal (Muiru *et al*., 2011; Wang *et al*., 2012). The causal fungus of SCLB is *Bipolaris maydis* (Y. Nisik. and C. Miyake) Shoemaker (Teleomorph *Cochliobolus heterostrophus* (Drechsler). Shoemaker; synonym = *Helminthosporium maydis* Nisikado) (Smith *et al*., 1970). SCLB is a remarkable disease affecting maize worldwide, potentially causing yield losses of up to 60% under severe conditions, based on susceptibility of the maize variety (Balint-Kurti and Pataky, 2024). This disease can be recognized by a variety of lesions, which are generally 1–6 mm wide and up to 2.5 cm long. Regardless of the type, lesions are brown to black (dark brown), oval, then generally fuse to form larger lesions in severe cases (Akonda *et al*., 2015). CLS is a common fungal disease of maize leaves that is widely distributed worldwide (Wang *et al*., 2022; Li *et al*., 2024). The causative agent of CLS has been identified as *Curvularia lunata* (Wakker) (Wang *et al*., 2019). It causes an appreciable reduction in maize production, with losses of over 60% in China maize planting area (Dai *et al*., 1996; Honghai *et al*., 1999). *C. lunata* infects maize leaves throughout their development, especially at the reproductive stage (Akonda *et al*., 2015; Bisht *et al*., 2016). Typical CLS symptoms begin as small chlorotic spots that gradually expand into round or oval lesions. These lesions are characterized by a chlorotic appearance, with a white or yellowish-brown center, a dark brown edge, and a surrounding yellowish halo (Dai *et al*., 1998).

For years, identification of these pathogens has depended on traditional morphological traits. But, these traits are often quite similar among many species, making these approaches insufficient for accurate identification (Khan *et al*., 2023). Recently, Polymerase Chain Reaction (PCR) amplification and subsequent sequencing of the internal transcribed spacer (ITS), translation elongation factor 1-alpha, calmodulin, beta-tubulin, glyceraldehyde-3-phosphate dehydrogenase, and mating type genes, have been extensively utilized for identification of these species (Manamgoda *et al*., 2012; Singh *et al*., 2021). The ITS serves as a universal barcode and appropriate to both *E. turcicum* and *B. maydis* (Nsibo *et al*., 2024).

In Egypt, the maize foliar fungal pathogens that have been a matter of considerable concern include *B. maydis, E. turcicum,* and *C. lunata*, their prevalence of which are remarkably increasing (Gouda, 1996; Hassan *et al*., 2015; Abdelsalam *et al*., 2022). These pathogens cause high-incidence epidemics under warm, humid, and wet conditions. These conditions are prevalent in Lower Egypt, especially in late-season crops (August planting) (El-Assiuty and El-Shafey, 1995). Maize leaf blight (MLB) has resulted in notable maize production losses, estimated at approximately 30% in the Northern Delta (Barakat *et al*., 2009). Earlier studies in Egypt confirm the presence of *B. maydis, E. turcicum,* and *C. lunata* contributing to the overall leaf spot complex (El-Shafey, 1970; Sabet *et al*., 1973). Additionally, earlier reports investigated cultural and morphological variations among these pathogens, physiological specialization on maize and sorghum hosts, and monitoring of virulence on maize cultivars (Diab *et al*., 1993; El-Naggar, 2006). Recently, many maize parents and hybrids have been assessed for their resistance to NCLB in various locations across Egypt. The obtained data indicated that crossing high-resistance maize cultivars produces appreciably higher levels of resistance to NCLB disease (Abdelsalam *et al*., 2022; Hawash *et al*., 2023).

In the laboratories, the sporulation of these fungal pathogens is a prerequisite for their pathogenicity, pandemic potential, and identification. Various natural substrates considerably influence the sporulation

behavior of these fungi (Senanayake *et al*., 2020). Species of *Bipolaris, Curvularia, Drechslera,* and *Exserohilum* typically exhibit abundant sporulation on naturally infected plant material (Miles and Wilcoxson, 1984; Sivanesan, 1987; Mattoo and Nonzom, 2022). Furthermore, their growth on cellulose substrates causes notable and substantial increases in sporulation (Pratt, 2006).

Climatic changes have led to an unprecedented rise in maize leaf diseases in Egypt. Furthermore, the symptoms of these diseases are complicated by host differences and variations in pathogen virulence. To our knowledge, the accurate molecular identification of maize leaf diseases has not yet been investigated in Egypt. Thus, the objectives of this study were to: (1) Isolate and identify the principal fungal species responsible for the leaf blight complex in Egyptian maize fields using morphological and molecular techniques; (2) determine a simple and efficient culture medium to enhance sporulation for subsequent pathological investigations; and (3) assess their pathogenic variability and virulence on maize and sorghum.

Materials and Methods

Sample collection

Maize leaf samples exhibiting typical symptoms of blight were collected from several governorates in Egypt, including Kafr el-Sheikh, Beni Suef, Nubaria, and Giza, during the growing season of 2022/2023. The infected leaves were identified based on disease symptoms such as chlorotic lesions, necrosis, and streaks characteristic of the MLB pathogens (Munkvold and White, 2016). Fifteen symptomatic maize leaf samples were selected based on their visible fungal infection symptoms, along with a subset of healthy maize leaves serving as controls for comparison.

Fungal isolation and culturing

Fungal isolates were obtained by surface sterilization of symptomatic leaves with 70% ethanol, followed by rinsing three times in sterile distilled water (SDW). Small leaf segments (5 mm^2) were aseptically excised using a sterile scalpel from the margins of the lesions, sterilized in 2% NaOCl for 30 sec, subsequently washed multiple times with SDW, and blotted dry. These segments were aseptically placed on the surface of Potato Dextrose Agar (PDA) petri plates and incubated at 27°C for 7 days. Following incubation, emerging fungal colonies were isolated and pure cultures were established using single-spore isolation technique. Pure cultures were preserved on PDA slants at 4°C for subsequent analysis (Singh *et al*., 2021).

Sporulation on culture agar media

This experiment aimed to identify an effective medium for sporulation of isolated fungi, which was easy to use and composed of simple components.

The following seven different culture media were tested to evaluate their effects on fungal sporulation (Pratt, 2006; Su *et al*., 2012):

1. Host Extract Agar (HEA): made by boiling surface-sterilized maize leaves (50 g) with distilled water (200 mL), filtering the extract, and mixing it with PDA at a 50% concentration before autoclaving.
2. Supplemented PDA with maize leaf pieces (MLP): surface-sterilized maize leaves (50 g) were cut into small 1-2 cm pieces using a surface sterilized scissor. These fresh leaf pieces were added directly to 1 L PDA before autoclaving.
3. Supplemented PDA with filter paper (FP): PDA medium was surface-amended by aseptically placing 20 sterile Whatman No. 1 FP strips (1 × 2 cm) onto the solidified agar in each plate. These strips were uniformly distributed across the surface to provide a consistent cellulose-rich substrate for fungal growth.
4. Calcium carbonate ($CaCO_3$) water agar (CWA): $CaCO_3$ 30 g; agar 20 g; distilled water 1L.
5. PDA Supplemented with cotton fabric (CF): A sterile piece of preshrunk 100% white cotton T-shirt fabric from COTTONIL MISR manufacturing (1 × 2 cm) was placed in PDA at a rate of 20 pieces/ plate.
6. Potato Dextrose Agar (PDA): Decoction of 200 g potato, 20 g dextrose, 20 g agar, 1 L distilled water.
7. Water Agar (WA): 20g agar, 1L distilled water.

Healthy maize leaves (5^{th}-6^{th} leaf stage) used in HEA and MLP were collected and surface-sterilized with sequential treatments of 70% ethanol (25 sec.) and 2% NaOCl (2 min.) followed by SDW rinses. The sterilized leaves were then cut into pieces (1-2 cm) using a surface sterilized sterile scissor before being incorporated into the media (Nwanosike and Mabagala, 2017). The pH of all media was

adjusted to 7.0 before autoclaving. A 5 mm fungal mycelial disc was aseptically cut out of an actively growing edge of a 7-day-old PDA culture using a sterile cork borer and placed at the center of each plate. Three replicate plates were prepared for each treatment (combination of fungus and medium). The inoculated plates were incubated at 27 °C for 10 d. The emerging spores were dislodged from the fungal colonies by flooding with 10 mL SDW containing a small amount of a surfactant (0.01% Tween-20), and the fungal colony surfaces were gently scraped using a sterile glass rod. The resulting spore suspension was filtered using a sterile cheesecloth to eliminate mycelial fragments, spore counts were determined *via* a hemocytometer, and inspected under a compound microscope (Laborlux S, Leitz, Wetzlar, Germany) at 400 x magnification. Nine independent counts were performed per treatment.

Pathogenicity experiments

Assessments of pathogenicity were conducted in the greenhouses of the Maize Diseases Department at the Plant Pathology Research Institute, Egypt, to confirm the causal relationship between the isolated fungal strains and the observed maize leaf disease symptoms. Additionally, cross-inoculation was performed on sorghum grains to assess the physiological specialization of the isolated fungi.

In the greenhouse, susceptible maize (*cv.* TWC310) and sorghum (*cv.* Giza15) were sown (4 seeds/pot) in 30 × 20 cm pots filled with a sterilized clay-loam soils (El-Naggar, 2006). Before inoculation, conidial suspensions of isolated fungi were prepared from 10-day-old cultures grown on MLP medium at 27 °C for a duration of 7 to 10 ds. Conidia were collected by inundating the plates with SDW and adding 0.2% solution of the surfactant Tween 20, followed by the gentle scraping of the surface using a sterile loop. The conidial concentration was determined using a hemocytometer and subsequently adjusted to a final concentration of 1×10^6 conidia/mL for each fungal species. Seedlings at the 5^{th}-6^{th} leaf stage were inoculated by spraying the conidial suspensions onto the leaves until runoff using a pump sprayer (Sun *et al.*, 2020). Control plants were sprayed with sterile distilled water and the surfactant agent. Four replicates were used for each fungal isolate and the control. Inoculated and control plants were covered with a polyethylene sheet for 24 h to maintain the desired humidity, after which the sheet was removed. Disease symptoms were observed daily for 14 ds post-inoculation (dpi). Disease severity was assessed following a 1-5 rating scale (Payak and Sharma, 1983) as follows:

1. Very mild infection: one to two or more scattered lesions are observed on lower leaves.
2. Light infection: A few lesions are present only on lower leaves.
3. Moderate infection: Abundant lesions exist on lower leaves, spreading up to middle and upper leaves.
4. Severe infection: Abundant lesions are present on lower and middle leaves, extending to upper leaves.
5. Intense severity: Abundant lesions are observed on almost all the leaves, with premature drying or necrosis of infected leaf tissue.

Furthermore, these scales were employed to calculate the percent disease index (PDI) using the formula reported by Wheeler (1969):

$$\text{PDI (\%)} = \frac{\text{sum of all ratings}}{\text{maximum disease rating} \times \text{total number of plants observed}} \times 100$$

To confirm Koch's postulates, symptomatic leaf tissues from inoculated plants were surface-sterilized, and pathogen re-isolation was performed on PDA. The re-isolated pathogens were then compared morphologically and microscopically to the original inoculated isolates.

Cultural and morphological variation

The fungal isolates were cultivated on PDA at 27°C for 10 ds to assess their cultural and morphological traits (Sun *et al.*, 2020; Singh *et al.*, 2021). Fungal growth rates were recorded at 24-h intervals until the plates were fully covered, using data from three replicates. Additionally, both the upper and lower surfaces of the culture plates were examined for colony color and texture. Furthermore, the size and septation of 30 conidia from each isolate were observed under a light microscope (Laborlux S, Leitz, Wetzlar, Germany). The isolates were initially identified through a comparative analysis of their morphological traits, including dimensions of conidia (*i.e.*, length and width) and the number of septa, with those documented in previous taxonomic investigations (Manamgoda *et al.*, 2014; Marin-Felix *et al.*, 2017).

Molecular identification

Growth plugs from actively growing margins of fungal isolates were inoculated into PDA and incubated for 7-8 d at 27˚C. Mycelia were carefully collected from the culture surface using a sterile spoon. Fungal DNA extraction was performed using the DNeasy Plant Mini Kit (Qiagen, Germany) following the manufacturer's instructions. The extracted DNA was quantified using UV spectrophotometry (Thermo Scientific NanoDrop 200 Spectrometer, USA), with concentration determined based on absorbance at 260 nm (A_{260}). The purity of the DNA extracts was assessed using the A_{260}/A_{280} and A_{260}/A_{230} ratios. DNA was stored at -20 ˚C until use.

The internal transcribed spacer region (ITS) of fungal isolates, encompassing the 5.8S rRNA gene (ITS1-5.8S-ITS2), was amplified and sequenced using the primer pair ITS1 (5`-TCCGTAGGTGAACCTGCGG-3`) and ITS4 (5`-TCCTCCGCTTATTGATATGC-3`) (White *et al*., 1990). Amplifications were conducted in a 25 µL reaction volume. Each reaction comprised 1 µL of genomic DNA (30 ng/µL), 1 µL of each primer (10 µM), 9.5 µL of nuclease-free water, and 12.5 µL of 2x Dream Taq Green Master Mix (Thermo Fisher Scientific, Waltham, USA). PCR amplification was performed using the following thermal cycling conditions: an initial denaturation step at 94˚C for 5 min, followed by 35 cycles of denaturation at 94˚C for 1 min, annealing at 55˚C for 1 min, and extension at 72˚C for 1 min. A final extension step at 72˚C for 10 min, was included. The amplified products were verified by electrophoresis in 1.5% (w/v) agarose gels stained with ethidium bromide. The purified PCR products were sequenced at the Macrogen Co. (Seoul, South Korea). The resulting sequences were compared against the complete nucleotide database at the National Center for Biotechnology Information (NCBI) using the Basic Local Alignment Search Tool (BLAST). ITS sequences of the isolates under investigation, along with relevant reference sequences retrieved from NCBI, were then aligned using Clustal W within MEGA 12.0.7, employing default settings. Phylogenetic relationships were determined from the ITS sequence data using MEGA software (Kumar *et al*., 2024). Maximum likelihood trees were constructed, applying the Kimura 2-parameter model, and branch support was assessed through bootstrap analysis with 1000 iterations.

Statistical analysis

Data on disease severity (%) and sporulation (spore count) were analyzed using analysis of variance (ANOVA). Percent disease severity data were arcsine square root transformed before analysis to normalize the distribution. All analyses were performed using GenStat (12th edition, VSN International Ltd.). Disease severity data were analyzed using one-way ANOVA to compare isolates. Sporulation data were analyzed with a two-way ANOVA to evaluate the effects of isolate, growth medium, and their interaction. Treatment means were separated using the Least Significant Difference (LSD) test at a 5% significance level ($p \leq 0.05$).

Results

Collection and isolation of fungal isolates

During 2022 and 2023 seasons, a total of 15 symptomatic samples exhibiting maize leaf blight-like symptoms were collected from four locations recognized as high to moderate hotspots for maize blight in Egypt. From seven single-spore isolates obtained from symptomatic leaves, two originated from Kafr el-Sheikh (Eg_23, and Eg_59), three from Nubaria (Eg_57, Eg_73, and Eg_88), one from Giza (Eg_60), and one from Beni Suef (Eg_52) as shown in Table 1.

Table 1: *Pathogenic variability among the fungal isolates collected from different regions of Egypt on maize and sorghum.*

Isolate	Location	Disease index (%)	
		Maize	Sorghum
Eg_23	Kafr el-Sheikh	21.25	0.0
Eg_52	Beni Suef	40	43.75
Eg_57	Nubaria	88.75	0.0
Eg_59	Kafr el-Sheikh	35	0.0
Eg_60	Giza	83.75	31.25
Eg_73	Nubaria	48.75	0.0
Eg_88	Nubaria	62.5	0.0
$LSD_{0.05}$		10.87	8.48

Sporulation on culture agar media

The sporulation of seven fungal isolates was evaluated using seven distinct culture media: HEA, MLP, FP, CF, CWA, PDA and WA. Spore production was quantified as the number of spores/ mL ($\times 10^4$) after 10 d of incubation at 27˚C, and the results are presented in Table 2.

Statistically significant differences in sporulation were observed among media, isolates, and their interactions ($LSD_{0.05}$: Media = 1.64, Isolates = 2.30, Media × Isolate = 5.85; $p \leq 0.05$). Among the seven tested media, the MLP medium produced the highest mean spore count at 48.3 × 10^4 spores/mL, followed by HEA (32.6 × 10^4) and FP (30.2 × 10^4). PDA yielded moderate sporulation (25.1 × 10^4), while CF, CWA, and WA were less effective, with mean spore counts of 15.9, 8.0, and 5.4 × 10^4 spores/mL, respectively.

Table 2: *Sporulation of fungal isolates from Egypt on different culture media.*

Isolate	Sporulation (10^4)							Mean
	HEA	MLP	FP	CF	CWA	PDA	WA	
Eg_23	62.0	85.1	54.9	34.2	16.6	32.1	9.2	42.0
Eg_52	17.8	41.6	34.6	11.3	2.5	20.4	1.7	18.6
Eg_57	25.8	32.2	26.5	14.5	9.9	27.4	6.6	20.4
Eg_59	35.0	39.8	26.2	9.8	8.7	27.6	6.1	21.9
Eg_60	22.7	41.4	24.4	15.3	5.8	21.9	3.8	19.3
Eg_73	36.0	45.5	19.8	10.7	4.1	22.2	3.6	20.3
Eg_88	28.6	52.6	25.1	15.6	8.4	24.1	6.7	23.0
Mean	32.6	48.3	30.2	15.9	8.0	25.1	5.4	
$LSD_{0.05}$	Media= 1.64 ; Isolates= 2.30; Media × Isolate= 5.85							

Where; Host extract agar (HEA); maize leaf pieces (MLP); filter paper (FP); Cotton fabric (CF); $CaCO_3$ water agar (CWA); Potato dextrose agar (PDA); Water agar (WA).

A significant interaction was observed between culture media and fungal isolates, as indicated by $LSD_{0.05}$ = 5.85. This finding demonstrated that the sporulation response of each isolate differed according to the specific growth medium. For instance, Eg_23 exhibited the highest sporulation on all media (mean = 42.0 × 10^4 spores/mL), particularly on MLP (85.1 × 10^4 spores/mL), and HEA (62.0 × 10^4 spores/mL). In contrast, Eg_52 had the lowest sporulation (mean =18.6 × 10^4 spores/mL) with its highest count on MLP recording 41.6 × 10^4 spores/mL. The remaining isolates (Eg_57, Eg_59, Eg_60, Eg_73, and Eg_88) demonstrated moderate sporulation with mean spore counts ranging from 19.3 to 23.0 × 10^4 spores/mL. Among these, Eg_88 achieved the highest spore count on MLP (52.6 × 10^4 spores/mL), whereas others like Eg_57 displayed a lesser response to this medium (32.2 × 10^4 spores/mL).

Pathogenic variability of fungal isolates

Two-week post-inoculation the maize plants, all treated leaves showed typical lesions, while the untreated controls had no symptoms (Table 1). The severity of the MLB varied significantly among isolates. Isolates Eg_57 and Eg_60 induced the highest severity, with disease incidence scores of 88.75% and 83.75%, respectively, followed by Eg_88 with a score of 62.5%. Moderate severity was noted for isolates Eg_73, Eg_52, and Eg_59, which scored 48.75%, 40%, and 35%, respectively. In contrast, isolate Eg_23 was the least virulent, with a score of 21.25%.

According to the morphology of lesions observed in the pathogenicity experiments, maize blight symptoms were classified into three categories. Type one symptoms, produced by isolate Eg_23, were characterized by small circular to elliptical yellowish necrotic lesions scattered across the leaf surface (Figure 1o). Type two symptoms, induced by isolate Eg_52, appeared as greyish or tan lesions that developed into elongated elliptical to spindle-shaped forms (Figure 1p). Type three symptoms expressed a differentiation in virulence. The moderately virulent isolate Eg_59 caused small, elliptical, or subrotund lesions with a tan center surrounded by reddish-brown margins (Figure 1r). In contrast, the highly virulent isolates (Eg_57, Eg_60, Eg_73, and Eg_88) produced typically larger elliptical or subrotund lesions that expanded more rapidly and formed irregular necrotic patches (Figure 1q, s, t, u).

On the other hand, only two isolates, Eg_52 and Eg_60, were pathogenic to the sorghum, causing leaf blight with disease index scores of 43.75% and 31.25%, respectively (Table 2). The symptoms on sorghum were categorized similarly to those on maize: isolate Eg_52 produced type two symptoms (elongated, nearly strip-like lesions), while isolate Eg_60 recorded type three symptoms (lesions that expanded more rapidly and formed irregular necrotic patches), as show in Figure 2.

Morphological characterization of pathogenic fungal species

The cultural traits of the seven pathogens, including growth patterns, colony coloration, and spore dimensions, were assessed for taxonomic identification using 10-day-old cultures grown on PDA medium. Based on these analyses, the fungal isolates were belonged to three distinct species. The resulting data are summarized in Table 3 and

illustrated in Figure 1.

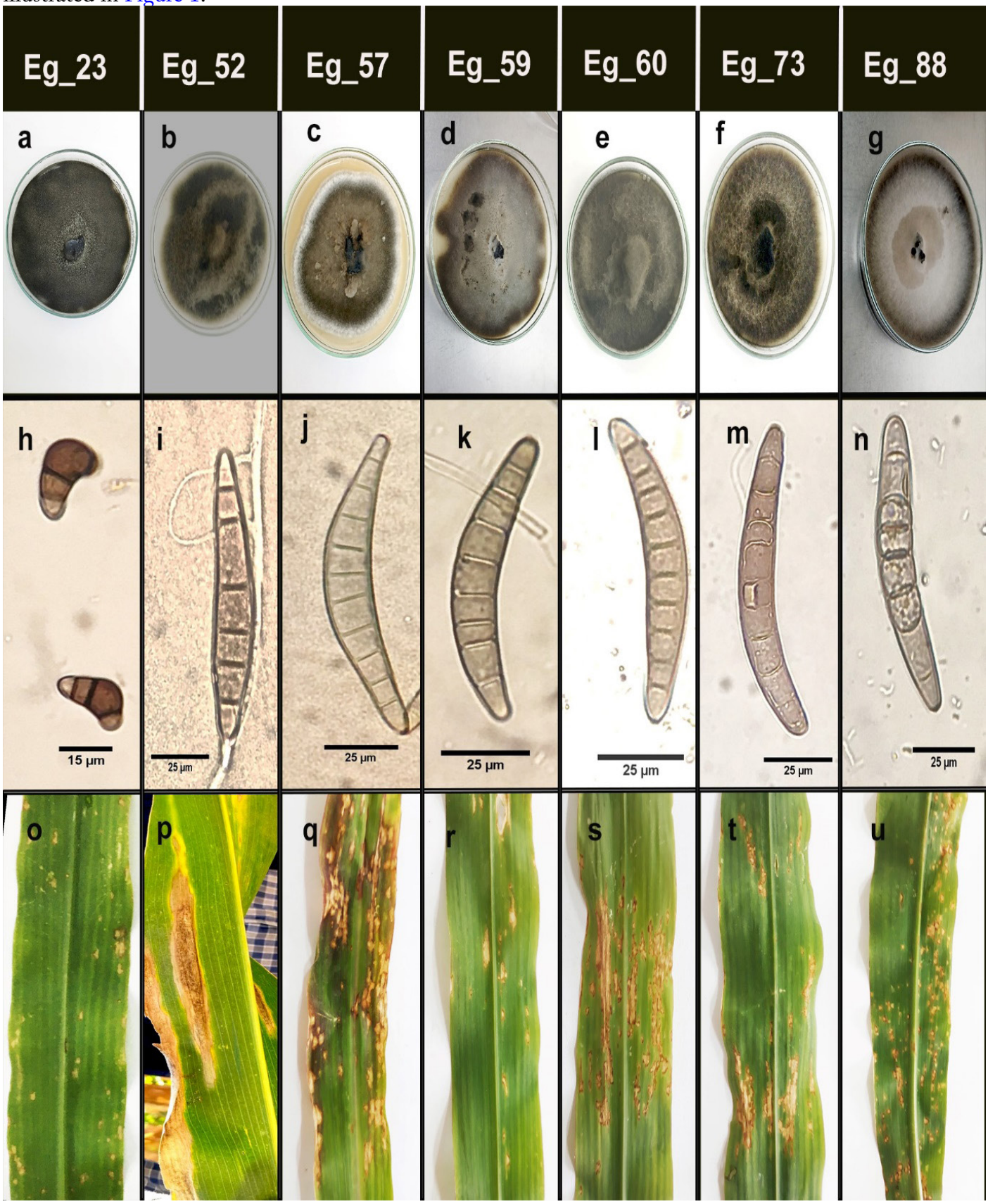

Figure 1: *Morpho-pathogenic characterization of fungal isolates associated with maize leaf blight in Egypt. (a-g) cultural variation of colonies grown on PDA until the plates were fully covered. (h-n) conidial morphology of each fungus, with scale bars indicating 15 µm or 25 µm. (o-u) foliar symptoms caused by each pathogen on maize leaves. Isolates were identified as: Curvularia lunata (Eg_23); Exserohilum turcicum (Eg_52), and Bipolaris maydis (Eg_57, Eg_59, Eg_60, Eg_73, Eg_88).*

Figure 2: *Symptoms of sorghum leaf blight caused by Eg_52 (a) and Eg_60 (b).*

Table 3: *Morphological and cultural characteristics of maize foliar diseases-causing fungal isolates.*

Isolate	Colony characterization	*Growth rate (mm/d)	**Size of Conidia (µm) and septations			
			Length (µm)	Width (µm)	Length width ratio	No. of septa
Eg_23	Dense, dark gray to grayish-black with powdery texture	17.6 ±0.18	27.7 ±2.7 (22.7-33.1)	10.4 ±1.7 (7.8-15.5)	2.7 ±0.3 (1.8-3.5)	3 ±0.0 (3-3)
Eg_52	Light grayish-white to dark gray/ black with a velvety-to-cottony texture.	4.7 ±0.06	77.2 ±12.9 (52.5-90.2)	13.0 ±2.4 (8.5-17.7)	6.0 ±1.0 (3.8-8)	5.9 ±0.8 (4-7)
Eg_57	Dark gray to black and the edges is white halo. Powdery to fluffy texture.	6.3 ±0.06	91.0 ±13.3 (70.1-117.7)	13.9 ±1.7 (11.4-17.3)	6.6 ±0.6 (5.1-7.7)	9.1 ±1.3 (5-11)
Eg_59	dark, smoky gray to black with powdery or velvety texture	9.9 ±0.09	87.9 ±9.2 (70.6-110.9)	13.0 ±1.4 (10.7-16.0)	6.8 ±0.6 (5.2-8.2)	8.8 ±0.9 (7-10)
Eg_60	Dark gray colonies with fluffy aerial mycelium	11 ±0.26	78.3 ±9.5 (67.4-99.1)	12.5 ± 1.2 (9.0-16.2)	6.3 ±1.1 (4.3-5.8)	8.2 ±1.1 (7-11)
Eg_73	Dark, smoky-gray to greenish-black. The texture appears velvety to slightly fluffy	10.3 ±0.15	89.8 ±13.4 (66.4-116.2)	11.5± 2.5 (7.1-14.3)	8.0 ±1.2 (6-9.9)	8.2 ±1.3 (7-10)
Eg_88	light, smoky-gray to greenish-gray with velvety to slightly powdery texture	13 ±0.13	80.9 ± 11.8 (64.8-101.7)	11.2 ± 2.9 (6.9-15.9)	7.6 ±1.8 (5-11.2)	8 ±0.8 (7-10)

*Where; *Fungal growth rates were recorded every 24-h until the plates were fully covered, and expressed as an average of 3 replications ± SD. * * The values in parentheses indicate the observed minimum and maximum range.*

The first species was represented by a single isolate (Eg_23), which conformed to the diagnostic features of *C. lunata* (Wakker) Boedijn. The colony displayed a dense structure with a dark gray to blackish-gray coloration and a powdery texture on its surface. It had the fastest growth rate among all tested isolates, measuring 17.6 mm/day. The conidia were characterized by a curved in shape with a swollen central cell. They were notably smaller than those of the other species (27.7 × 10.4 µm), with an average length-to-width ratio of 2.7 and typically exhibited 3-septate (Figure 1a, h). The second species comprised single isolate (Eg_52), consistent with the description of *E. turcicum* (Passerini) K.J. Leonard and E.G. Suggs. The colony initially appeared light grayish-white and then developed dark gray to black, with a velvety to cotton-like texture. This isolate demonstrated a remarkably slow growth rate of 4.7 mm per day. It produced large, pale brown conidia that were straight to slightly curved, featuring a distinctly protruding hilum. The conidial dimensions averaged 77.2 × 13.0 µm, with septation ranging 4-7 (mean of 5.9) (Figure 1b, i). The remaining five isolates (Eg_57, Eg_59, Eg_60, Eg_73, and Eg_88) were all identified as *B. maydis* (Y. Nisik. and C. Miyake) Shoemaker, which was the predominant species observed in this study. The colonies displayed a color range from light smoky-gray to greenish-black, with textures that varied from powdery and velvety to fluffy (Figure 1c-g). The growth rates of this group were categorized as moderate to fast, with measurements from 6.3 (Eg_57) to 13.0 (Eg_88) mm per day. All *B. maydis* isolates produced relatively large conidia, which were geniculately curved, oblong to fusiform, pale olive-brown, and multi-septate. The conidia were notably substantial in size, with mean lengths varying from 78.3 µm (Eg_60) to 91.0 µm (Eg_57), and mean widths from 11.2 µm (Eg_88) to 13.9 µm (Eg_57). These conidia were highly septate, typically containing between 5 and 11 septa on average (Figure 1j-n).

Phylogenetic analysis

Identification of the pathogenic fungi at the molecular level was verified through phylogenetic analysis of ITS region sequences, with species assignments further validated by BLASTn analysis against the NCBI GenBank database. The analysis results indicated that five isolates (Eg_57, Eg_59, Eg_60, Eg_73, and Eg_88) exhibited >99% identity with *B. maydis*. In contrast, isolate Eg_23 matched *C. lunata* with 99.9% identity, while isolate Eg_52 showed high sequence similarity of 99.6% to *E. turcicum*. To further support these findings, the obtained ITS sequences from pathogenic isolates Eg_23, Eg_52, Eg_57, Eg_59, Eg_60, Eg_73, and Eg_88 were submitted to NCBI GenBank and assigned the following accession numbers: OQ862267, OQ862341, OQ862319, OQ862320, OQ862321, OQ862322, and OQ862323, respectively.

The ITS sequences from seven pathogenic fungi collected in this investigation, along with reference sequences of helminthosporium fungi, including 12 *B. maydis,* 6 *C. lunata,* and 6 *E. turcicum*, as well as an outgroup (*Alternaria alternata*) originating from GenBank, were used to construct a phylogenetic tree. The final ITS alignment file had 32 taxa and 630 characters, of which 103 were parsimony informative, representing 16% of the total characters. The maximum likelihood tree in Figure 3 showed that the seven pathogenic isolates under investigation were clustered into three distinct and strongly supported clades, each corresponding to a particular species. Five isolates (Eg_57, Eg_59, Eg_60, Eg_73, and Eg_88) constitute a well-supported clade alongside a sequence from the ex-type culture *B. maydis*. Isolate Eg_23 shared an identical sequence with the cultures of *C. lunata* and grouped with them in a highly supported clade. Likewise, isolate Eg_52 was found to cluster tightly with *E. turcicum*, sharing identical ITS sequences with ITS reference strains. All major clades received bootstrap support values of 100%, indicating strong confidence in species-level resolution. So, the ITS-based phylogeny validated the morphological identifications and clearly separated the isolates into *B. maydis, C. lunata,* and *E. turcicum*.

Discussion

Maize leaf blight is a remarkable biotic threat to maize production worldwide including Egypt, resulting in a decrease in quantity and quality of its grain (Kumar *et al*., 2022). Accurate characterization of the causal agents infecting maize leaves in Egypt is crucial for planning effective management strategies to address economic losses and for clearly understanding the severity of these diseases. In the present investigation, 15 symptomatic samples were collected from four agricultural locations known to be high to moderate hotspots for maize blight in Egypt. Seven single-spore fungal isolates were collected and subsequently identified as three distinct species: *C. lunata, E. turcicum*

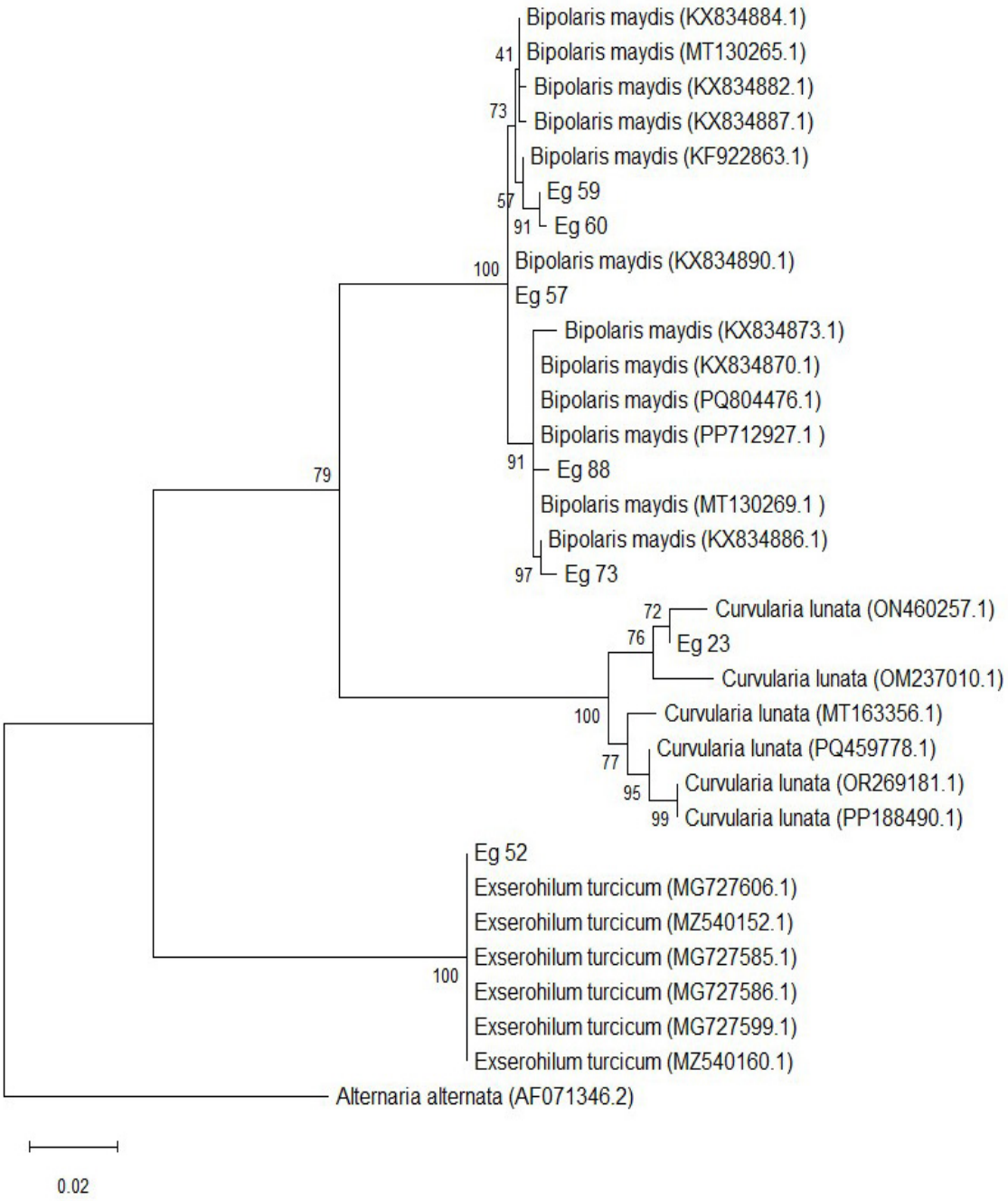

Figure 3: *Maximum likelihood phylogenetic tree reconstructed from ITS sequences of the seven Egyptian fungal isolates causing maize leaf bligh within related reference sequences obtained from GenBank. Numbers on the branching points are ≥40% bootstrap values from a bootstrap test of 1000 replicate. The scale bar refers to number of nucleotide substitutions per site. The tree was rooted with Alternaria alternata.*

and *B. maydis*, which were the primary dominants. These obtained results are consistent with earlier studies on the causes of MLB, where these species have been identified as primary pathogens affecting maize globally (de Sousa Ramos *et al*., 2024; Nsibo *et al*., 2024; Abera *et al*., 2025). Maize leaf blight had been recorded to be widespread in the northern and northwestern areas of Egypt, due to favorable weather conditions (El-Assiuty *et al*., 1987). Therefore, these areas are considered an appropriate location to address MLB (Abdelsalam *et al*., 2022; Hawash *et al*., 2023). The presence of these pathogens together in Egyptian maize fields highlights the risk of mixed infections and the challenges associated with managing these diseases.

The efficient production of conidia is essential for conducting pathological studies, screening for resistance, and researching fungal biology. In this study, evaluation of sporulation across the tested culture media indicated that the composition of the culture medium appreciably influenced spore production in

the fungal species. Maize leaf–based media (MLP and HEA) significantly enhanced conidia production, while nutrient-poor or inert substrates such as WA and CWA supported minimal spore formation compared to PDA medium. These findings indicated that host-derived components were crucial for stimulating sporogenesis of MLB pathogens. The obvious effectiveness of MLP and HEA may be attributed to complex matrix of nutrients and surface structures that mimic the natural host environment, stimulating conidiogenesis in these pathogenic fungi (Su *et al*., 2012). This aligns with previous studies reporting that host-derived substrates can stimulate spore production in plant-pathogenic fungi by mimicking natural host conditions (Garraway, 1975; Garraway and Evans, 1977; Harrison, 1980; Sivanesan, 1987). Filter paper (FP) as a supplement also supported relatively high sporulation. Cellulose-based materials, such as FP, may mimic the physical structure of plant cell walls (Ou *et al*., 2025). This similarity provided a substrate for enzymatic activity and a surface for conidiophore formation, which encouraged sporulation rather than solely promoting vegetative growth (Pratt, 2006). The moderate performance of PDA is consistent with its well-established role as a standard, nutrient-rich medium for general fungal cultivation but lacked the host-specific cues present in MLP and HEA. In contrast, CF, CWA, and WA were remarkably yielded the lowest sporulation. Their poor performance may be due to their minimal nutrient content, which limits fungal growth and sporulation (Dhingra and Sinclair, 1985; Mattoo and Nonzom, 2022).

The appreciable interaction between media type and fungal isolates reflected the distinct physiological differences among the fungal species and even among isolates of the same species. *C. lunata* (Eg_23) exhibited consistently high sporulation across all tested media which is consistent with its established saprophytic capacity and rapid growth observed during morphological assessment. In contrast, *E. turcicum* (Eg_52) displayed the slowest growth and the lowest spore production, reflecting a more fastidious physiological profile. The differential responses observed among the five *B. maydis* isolates indicated the genetic variability in their adaptation to environmental and nutritional conditions, which may influence their potential for field adaptability and dissemination (Kumar *et al*., 2025). Sporulation is a complex process influenced by both environmental and genetic factors, which may differ among isolates (Su *et al*., 2012). Additionally, Pratt (2006) noted variations in sporulation among fungal species and isolates.

The pathogenicity assessments conducted on maize revealed significant differences in virulence among the isolates. The five *B. maydis* isolates displayed moderate to high levels of aggressiveness, as indicated by disease incidence scores that varied between 35 and 88.75. Additionally, the symptoms were categorized from small, elliptical lesions to large, rapidly expanding ones. Our results align with the previous surveys conducted in China and India, which identified *B. maydis* as a dominant MLB pathogen and a highly virulent agent responsible for severe MLB outbreaks (Guo *et al*., 2016; Sun *et al*., 2020; Singh *et al*., 2021).

Interestingly, the single *E. turcicum* (Eg_52) induced moderate severity on maize. Although, *E. turcicum* is usually highly destructive, this moderate virulence may result from isolate characteristics, host genotype, or environmental conditions during testing (Perkins and Pedersen, 1987). The *C. lunata* isolate Eg_23 expressed the lowest virulence with small, localized necrotic lesions. Previous studies have identified *C. lunata* as a weak pathogen or a secondary invader associated with milder symptoms (Manamgoda *et al*., 2014; Wang *et al*., 2022).

Notably, only two isolates, *E. turcicum* (Eg_52) and *B. maydis* (Eg_60), had the ability to infect sorghum, producing symptoms similar to those observed on maize (type two for Eg_52 and type three for Eg_60). This finding has critical implications for Egyptian agricultural practice, where the potential for both pathogens to persist on sorghum could raise inoculum levels, complicating disease management in mixed-cropping systems (Langenhoven *et al*., 2020; Nsibo *et al*., 2024). Additionally, these results highlighted the broader host range of these pathogens. The observed cross-infectivity aligns with several previous studies demonstrating that *E. turcicum* and specific strains of *B. maydis* strains can infect both maize and sorghum (El-Shafey *et al*., 1982; Tang *et al*., 2015; Cui *et al*., 2024).

The observed MLB symptoms which were classified into three types based on lesion morphology demonstrated an obvious correlation with the specific fungal species present. Additionally, they provided a practical framework for field diagnostics and disease

monitoring. Type one symptoms appeared as small, yellowish necrotic lesions induced by *C. lunata*, characteristic of leaf spot. Type two symptoms associated with *E. turcicum*, were presented as elongated, spindle-shaped lesions; a classic diagnostic feature of NCLB. In contrast, the lesions produced *by B. maydis* (Type three) were more variable, generally larger, and more likely to form irregular necrotic patches, characteristic of SCLB (Payak and Sharma, 1983; Guo *et al*., 2016; Singh *et al*., 2021). These symptom types demonstrated the varying pathogenicity of the identified fungal species and could support an early detection and targeted management of MLB in affected regions.

Morphological and molecular characterization unambiguously confirmed the identity of the isolates. The ITS phylogeny clustered all isolates into well-supported clades corresponding to *B. maydis, C. lunata,* and *E. turcicum*, with >99% sequence identity to reference strains. The species-level resolution was confirmed by high bootstrap values of 100%. This result validated the morphological identifications based on conidial shape, septation, and colony appearance (Munkvold and White, 2016). The agreement between these methods provides a reliable basis for subsequent pathological analyses. Previous studies have demonstrated the effectiveness of ITS-based phylogenetic approaches in resolving relationships among helminthosporioid fungi (Manamgoda *et al*., 2012; Hernandez-Restrepo *et al*., 2018; Sun *et al*., 2020; Kumar *et al*., 2023). The observed concordance between morphological and phylogenetic data in this study further supports the reliability of pathogen identification.

Conclusions and Recommendations

Our findings indicate that MLB in Egypt is induced by a complex of at least three fungal species: *B. maydis, E. turcicum,* and *C. lunata,* with *B. maydis* being the most commonly isolated and often the most virulent. Phylogenetic analysis validated the morphological identifications and established a molecular baseline for future surveillance. The recognition of fungal isolates that exhibit pathogenicity on both maize and sorghum raises concerns about the role of alternative hosts in the disease epidemiology. Future studies are recommended to explore the genetic basis of virulence and host specificity, especially for *B. maydis*, and assess resistant crop varieties to improve disease management.

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Novelty Statement

This study provides the first definitive morpho-molecular identification of the maize foliar blight complex in Egypt, establishing *Bipolaris maydis* as the predominant and most aggressive pathogen, alongside with *Exserohilum turcicum* and *Curvularia lunata*. The novelty lies in validating a simple, enhanced medium through supplementing PDA with maize leaf pieces (MLP) as a highly effective and host-simulating substrate for mass sporulation of Egyptian isolates, providing a practical and optimized tool for inocula production. Furthermore, this study reports a critical epidemiological finding; mainly the novel cross-pathogenicity of a local *B. maydis* isolate to sorghum, revealing an expanded host range that complicates disease management and necessitates revised control strategies in Egyptian agriculture.

Author's Contribution

Eihab Mohamed Taha: Conceptualization, methodology, investigation, formal analysis, data curation, writing original draft preparation.
Elhamy M. El-Assiuty: Supervision, resources, validation, writing review and editing.
Zeinab M. Fahmy: Investigation, methodology, validation, writing review and editing.
Doaa A. Kafsheer: Formal analysis, investigation, data curation, visualization, software.

Ethical approval
This study did not involve human participants or animals. All plant materials (maize and sorghum) used in this research were cultivated and handled in accordance with standard agricultural practices for scientific research.

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Generative AI and AI-assisted technology statement

The authors declare that no Generative AI or AI-assisted technologies were used in the writing of this manuscript or in the creation of its intellectual content.

Conflict of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this study.

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