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Research Article

Characterization of Stem-End Rot Pathogens and Field Evaluation of Systemic Fungicides in Mango Orchards of Punjab, Pakistan

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ABSTRACT

Stem-end rot (SER) is a major postharvest disease that limits mango (*Mangifera indica* L.) production and export potential in Pakistan. This study developed a sustainable SER management framework by integrating pathogen characterization with chemical control strategies. Fungal isolates collected from six districts of Punjab were identified morphologically and molecularly as *Lasiodiplodia theobromae*, *Colletotrichum gloeosporioides*, *Curvularia spicifera*, and *Alternaria alternata*. Pathogenicity assays confirmed their role in SER, with *L. theobromae* producing the largest lesions (74.6 mm). *In vitro* fungicide screening revealed fludioxonil as the most effective compound, inhibiting mycelial growth by 84.6-94.4% across all pathogens at 500-2000 ppm. Field trials showed that fludioxonil at 2000 ppm reduced SER incidence and severity by 87.1% and 92.1%, respectively, compared to untreated controls (71.3% incidence). Azoxystrobin and difenoconazole demonstrated moderate efficacy, while fluoxastrobin was the least effective. Phylogenetic analysis indicated close genetic relatedness between Pakistani isolates and those from India and China, suggesting regional pathogen dispersal. These results establish fludioxonil as a highly effective fungicide for SER management under Pakistani conditions. To mitigate resistance development and enhance sustainability, its use should be integrated with cultural practices and biocontrol agents. Overall, this study provides a science-based, region-specific integrated disease management model to improve mango quality, reduce postharvest losses, and strengthen Pakistan's export competitiveness.

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Introduction

Mango (*Mangifera indica* L.) is among the most economically important tropical fruit crops worldwide and represents a major component of agricultural exports in many developing countries (Bini et al., 2024). Pakistan ranks as the fourth-largest mango producer globally and plays a pivotal role in sustaining rural livelihoods and generating foreign exchange earnings (Kamal et al., 2022). Despite its high market value and strong export potential, the Pakistani mango industry experiences substantial postharvest losses, primarily due to stem-end rot (SER) (Ahmad et al., 2025), a destructive fungal disease that severely compromises fruit quality, shelf life, and marketability (Obedgiu et al., 2025). SER typically appears as a light-brown discoloration near the peduncle that rapidly expands into large, dark necrotic lesions (Harsh,

2025). The disease progresses internally, leading to tissue maceration, pulp softening, and eventual fruit breakdown, often without conspicuous external symptoms until advanced stages of decay (Karunanayake et al., 2020; Adikaram et al., 2024; Peja et al., 2025).

Globally, SER is caused by a complex of fungal pathogens, predominantly *Lasiodiplodia theobromae*, *Botryosphaeria dothidea*, *Colletotrichum gloeosporioides*, *Neofusicoccum parvum*, and *Diaporthe pseudomangiferae* (Coelho et al., 2022; Li et al., 2023; Yeo et al., 2024). These fungi establish latent infections during the flowering stage by asymptomatically colonizing inflorescences and pedicel tissues (Viret and Gindro, 2024). They persist endophytically within vascular and cortical tissues, with symptom expression triggered during fruit ripening when host physiological changes create conditions favorable for pathogen proliferation (Johnson et al., 2019; Harish et al., 2023; Zhan et al., 2023). Notably, levels of fungal colonization detected approximately 11 weeks after flowering show a strong correlation with SER incidence at harvest, emphasizing the importance of early disease monitoring (Johnson et al., 2019; Bill et al., 2021; Slimani et al., 2024).

Economically, SER contributes significantly to the estimated annual global mango losses of 8.6 million metric tons, with an approximate value of USD 355.2 million (Le et al., 2022; Ahmad et al., 2025). In Pakistan, these losses are further aggravated by inadequate postharvest handling practices, including injurious manual harvesting and poor orchard sanitation, which facilitate pathogen entry and inoculum accumulation (Imran et al., 2025). Commercially important cultivars such as Sindhri and Chaunsa exhibit high susceptibility, particularly in older orchards where pathogen pressure increases over time (Zhan et al., 2023; Hammad et al., 2025). Consequently, SER management has traditionally relied on the prophylactic application of systemic fungicides during the pre-harvest period (Kumar and Choudhury, 2025).

Given these challenges, accurate pathogen identification and rigorous fungicide evaluation are essential initial steps toward developing sustainable SER management strategies (Ceresini et al., 2024). Molecular diagnostic approaches, particularly internal transcribed spacer (ITS)-based sequencing, enable precise species identification and phylogenetic resolution of SER-associated pathogens (Choudhary et al., 2021).

This study addresses critical gaps in the management of SER within Pakistan's mango production systems (Akhtar et al., 2025). The present investigation provides a comprehensive characterization of SER-associated fungal species across major mango-growing districts of Punjab using integrated morphological and molecular approaches. The pathogenicity of dominant isolates was validated through Koch's postulates, and their sensitivity to four systemic fungicides viz., fludioxonil, azoxystrobin, difenoconazole, and fluoxastrobin was assessed under both laboratory and field conditions. By linking pathogen identity with fungicide efficacy, this study establishes a scientific basis for rational chemical management of SER in Pakistan, with direct implications for reducing postharvest losses and improving mango export quality.

Materials and Methods

Survey, sample collection, and processing

Mango fruits at physiological maturity were collected from five orchards in each of six major mango-growing districts of Punjab, Pakistan, Bahawalpur, Rahim Yar Khan, Multan, Khanewal, Muzaffargarh, and Lodhran (Hammad et al., 2025). The selected orchards were located 5-10 km apart. From each orchard, 100 fruits were randomly harvested from 20 trees.

Fruit surfaces were disinfected using 2% sodium hypochlorite, thoroughly rinsed with tap water, and air-dried for 30 min (Yeo et al., 2024). Following surface disinfection, the fruits were initially stored at 14.5 ± 0.5°C and 80 ± 2% relative humidity (RH) for 14 days to simulate cold storage conditions. Subsequently, the fruits were transferred to a ripening room maintained at 31 ± 5°C and 70 ± 5% RH to induce symptom development (Singh et al., 2025).

Thereafter, the inoculated fruits were incubated at 26 ± 2°C and 55 ± 2% RH for 10 days to ensure uniform disease development and lesion measurement. Control fruits were maintained under identical environmental conditions (dos Santos et al., 2024).

Disease parameters assessment

Disease incidence and severity were assessed using standard protocols (Chambers et al., 2025). Disease incidence was calculated as the percentage of infected fruits per orchard by dividing the number of symptomatic fruits by the total number of fruits sampled (Khaskheli, 2020; Zwane et al., 2023).

$$\text{Disease Incidence} = \frac{\text{Number of Infected Fruits}}{\text{Total Number of Sampled Fruits in Orchard}} \times 100$$

Disease severity was assessed using the 0-6 scale of Alvindia and Acda (2015), where 0 = no discoloration and 6 = complete fruit surface discoloration due to stem-end rot (Peja et al., 2025). These scores were used to compute a percentage disease severity index as follows:

$$\text{Disease Severity Percentage} = \frac{\sum \text{of SeverityScores}}{\text{MaximumPossibleSeverityScore} \times \text{TotalNumberofFruits}} \times 100$$

Pathogen isolation, purification, and morphological identification

Fungal pathogens associated with stem-end rot were isolated from symptomatic mango tissues following the procedure described by Yeo et al. (2024). Small tissue sections (≈2 mm) excised from the margins of active lesions were surface-sterilized, plated onto Potato Dextrose Agar (PDA), and incubated at 25°C. Emerging fungal colonies were subcultured repeatedly to obtain pure cultures (Malhotra et al., 2025). Morphological identification was carried out based on colony characteristics, hyphal features, and conidial morphology (shape, size, and septation) observed under a light microscope (up to 100× magnification), using the taxonomic keys of Barnett and Hunter (1998), as described by Kaouache et al. (2025).

Pathogenicity test

The pathogenicity of all fungal isolates was confirmed by inoculating surface-sterilized, asymptomatic ripe mango fruits in accordance with Koch's postulates (Abdurrehman et al., 2024). A 5-mm mycelial plug obtained from a 7-day-old PDA culture was inserted into a wound made at the peduncle region, while sterile PDA plugs were used as controls (Dutra et al., 2025). Inoculated fruits were incubated at 26 ± 2°C and 55 ± 2 % relative humidity for 10 days (Sbodio et al., 2024; Yeo et al., 2024). Fruits were examined daily for the development of stem-end rot symptoms (Ghattamaneni et al., 2020). The pathogen was re-isolated from symptomatic tissues, and its identity was confirmed by morphological comparison with the original isolate (Oğuz et al., 2025). Only isolates that consistently reproduced disease symptoms and were successfully re-isolated were considered pathogenic (Brglez et al., 2024).

Extraction of fungal genomic DNA

Genomic DNA was extracted from 7-day-old mycelial cultures of pathogenic isolates using the cetyltrimethylammonium bromide (CTAB) method (Galsurker et al., 2020; Saikia et al., 2025). Mycelia were harvested from potato dextrose broth, lyophilized, and ground into a fine powder prior to DNA purification (Ameen et al., 2021). The extracted DNA was resuspended in nuclease-free water and used for downstream molecular analyses (Sasi et al., 2023).

The internal transcribed spacer (ITS) region of fungal ribosomal DNA was amplified using the universal primers ITS4 and ITS5 (White et al., 1990; Hiruma et al., 2023). Polymerase chain reaction (PCR) was performed under standard thermal cycling conditions, and the resulting amplicons were resolved by agarose gel electrophoresis (Yeo et al., 2024). Bands were visualized using a Bio-Rad Gel Doc XR system equipped with Quantity One software for documentation and analysis (Ekanayake et al., 2019; Phuphisut et al., 2024).

Nucleotide sequencing

Purified PCR products were subjected to Sanger sequencing by Macrogen Inc. (Seoul, South Korea) (Elsababty et al., 2022). Raw sequence data were manually edited to ensure quality, and consensus sequences were submitted to GenBank using the BankIt submission tool (Truong Nguyen et al., 2021). Accession numbers were assigned by the National Center for Biotechnology Information (NCBI) following sequence validation (Sayers et al., 2022).

***In vitro* evaluation of different fungicides against stem-end rot pathogens of mango**

The efficacy of four systemic fungicides, namely fludioxonil, azoxystrobin, difenoconazole, and fluoxastrobin (Table 1), was evaluated against SER-associated fungal pathogens using the poisoned food technique (Nene and Thapliyal, 1982; Ekabote et al., 2024). The fungicides were tested at concentrations of 500, 1000, 1500, and 2000 ppm, with each treatment replicated three times (Dinani et al., 2023). Mycelial growth inhibition was assessed after 7 days of incubation at 26 ± 2°C, and percent inhibition was calculated relative to the untreated control (Feng et al., 2019; Mannai et al., 2022).

$$\%\ \text{growth inhibition}(\text{PI}) = \frac{\text{C} - \text{T}}{\text{C}} \times 100$$

C = colony growth in the control plate
T = colony growth in treated plate.

Table 1. Commercial fungicide formulations used for the management of SER in mango.

Sr. No.	Brand name	Company	Active ingredient	Formulation
1	Scholar	Syngenta	Fludioxonil	Suspension Concentrate
2	Epical	Jaffer group of Companies	Azoxystrobin	Suspension Concentrate
3	Difenoconazole	FMC	Difenoconazole	Emulsifiable Concentrates
4	Avito	Arysta Life Science	Fluoxastrobin	Suspension Concentrate

Statistical analysis

In vitro experiments, including fungicide efficacy assays, were conducted using a Completely Randomized Design (CRD) with three replications per treatment (Abrahim et al., 2025). Field experiments assessing fungicide performance against naturally occurring stem-end rot were laid out in a Randomized Complete Block Design (RCBD) with five replications. The data were subjected to analysis of variance (ANOVA), and treatment means were separated using Tukey's Honestly Significant Difference (HSD) test at $p \leq 0.05$. All statistical analyses were performed using Statistix 8.1, while graphical presentations were generated using GraphPad Prism (Gray et al., 2025).

Results

Field survey of SER in mango

A field survey conducted across 24 sublocations in six major mango-growing districts of Punjab, Pakistan, Bahawalpur, Rahim Yar Khan, Multan, Lodhran, Khanewal, and Muzaffargarh, revealed significant spatial variation in the incidence and severity of SER (Tables 2, 3, 4, 5, 6, 7), indicating pronounced regional differences in disease dynamics (Atiq et al., 2025).

Table 2. Georeferenced data of surveyed orchards and SER incidence and severity (%) in Bahawalpur district.

Sub	Orchard Name	Latitude (N)	Longitude (E)	(%) Disease	(%) Disease
Bahawalpur	Jalwana mango Farm,	29.2202	71.4459	17	8
	Awaisi Mango orchard,	29.3070	71.5406	16	7.2
	Asif mango farm Dera Masti,	29.3309	71.5705	15	7.4
	Shahab Mango Farm	29.3111	71.5550	13	6
	Mian Ejaz Muhammad Awaisi	29.3072	71.5425	20	8.6
Ahmedpur East	Nao lakha Fruit Farm	29.2670	71.0336	11	4.55
	Hassan Fruit Fram	29.1539	71.2961	9	4
	Aziz Fruit Farm Basti Khokhran	29.1611	71.3222	12	5.4
	Tariq Hameed Mango Farm	29.1545	71.3042	11	3.6
	Bahawal Mango Farm DNS	29.1076	71.2819	17	6.4
Yazman	Chaudary Mango Farm 23 DNB	29.0970	70.9425	12	3.8
	Chaudhary Shahid Mango Farn 26DNB	29.0624	71.4072	9	3
	Chaudhary Imtiaz Mango Farm 21DNB	29.0830	70.8435	12	5.2
	Judge Wala Bagh 22 DNB	29.0744	71.5082	11	3.8
	Chaudhary Abid Mango Farm 17 DNB	29.0880	70.7435	15	5
Khair Pur Tamewali	Riaz Peer Zada Mango Farm Qaiem Pur	29.7308	72.3760	13	5
	Bhatti Mango Farm Lal Sunhara	29.5808	72.2211	15	5.4
	Sultan Khokhar Mango Farm Lal Sunhara	29.7226	72.3750	12	2.82
	Hafiz Jahangir Mango Farm Lal Sunhara	29.7323	72.3845	13	5
	Alam Geer Mango Farm Pull Hamdani	29.7323	72.3845	16	5.6
Hasil Pur	M. Ali Mango Farm Moza Muhammad Pur	29.7553	72.4728	21	8.4
	Rehan Mango Farm Chak 156 Murad	29.7553	72.4729	18	5.6
	Iqbal Marali Mango Farm Jamal Pur	29.6813	72.4078	17	6.6
	Muhammad Khan Marali Jamal Pur	29.6501	72.3531	20	7.2
	Chaudhary Zeshan Mango Farm Jamal Pur	29.7323	72.6038	14	5.6

Table 3. Georeferenced data of surveyed orchards and SER incidence and severity (%) in Rahim Yar Khan district.

Sub Locations	Orchard Name	Latitude (N)	Longitude (E)	(%) Disease Incidence	(%) Disease Severity
Rahim Yar Khan	Maitla Mango Orchard Chak 113P, RYK	28.3483	70.2811	13	6
	Mian Shazaib Mango Farm,	28.4079	70.0995	14	6
	Mohsin Mango Orchard, Bangla Manthar	28.2048	70.2413	12	4
	Kashif Mango Farm, Canal Road, RYK	28.4427	70.3033	10	3.8
	Rahim Mango Farm, Abad Pur, RYK	28.6135	70.2842	11	3.8
Khan Pur	DC Mango Farm, Chak 7P	26.2879	64.4512	17	7.6
	Rahmatullah Mango Orchard, Chak 4P	28.6642	70.6924	19	5.6
	Chaudhary Ibrahim Mango Orchard	28.40440	69.9405	20	7.2
	Muzzafar Hussain Orchard	28.6507	70.6937	14	5
	Shahid Shah Mango Orchard	28.9613	70.7255	11	3.6
Sadiqabad	Indrahr Mango farm	28.3283	69.8624	14	5.2
	Muhammad Anwar Kamal Mango Farm	28.2739	70.2003	17	5.8
	Mahar Mango Farm	28.3863	70.3646	11	4.4
	Javeed Seth Farm, Akhtherabad	28.9514	70.7264	15	4.4
	Jam nadir mango orchard	28.1655	70.1041	10	3.2
Liaqat Pur	Qazi Siraj Ahmed, Allah Bad	28.5653	70.5257	19	5.6
	Ch. Akhtar Mango Farm	28.9508	70.7252	15	4.4
	Israr Khan Mango Orchard, Allah Bad	28.9481	70.8226	17	7.2
	Ch. Rustum Mango Orchard	28.9308	70.7452	13	4.4
	Asif Member Mango Farm	28.9482	70.2826	10	3.2

Table 4. Georeferenced data of surveyed orchards and SER incidence and severity (%) in Multan district.

Sub Locations	Orchard Name	Latitude (N)	Longitude (E)	(%) Disease Incidence	%) Disease Severity
Multan	Hamid Pir Murkha Farm	30.3794	71.5860	21	7.2
	Alizai Mango Farm, Nawab Pur, Multan	30.3260	71.4890	18	6
	Chaudhary Mango Farm, Multan	29.8831	71.3579	20	8.2
	MRC, Multan	29.8830	71.3545	19	7.6
	Latifabad Mango Farm	30.3412	71.4887	17	6.6
Jalal Pur Pirwala	Chanab Mango Farm, Jalal Pur Pirwala	29.4142	71.3241	12	4
	Organic Mango Farm	28.3957	70.3792	18	7.6
	M. Sajid Araein, Mouza Umer Pur	29.5179	71.1674	11	4.4
	Shafeeq Ahmed Mango Orchard	29.5163	71.1544	17	7
	Manzoor Hussain mango farm	29.5074	71.1577	20	7.6
Shujabad	Mango Research Station Shujabad	29.8831	71.3579	14	5.6
	Ashfaq Ahmad Khakhi mango Farm	29.7089	71.2496	10	3.8
	Khakhi Mango farm	29.8947	71.3547	17	6.6
	Hassan Khakhi mango farm	29.8957	71.3430	19	6
	Asghar mango farm	29.9015	71.3492	12	3.6

Morphological characterization of fungi isolated from SER–affected mangoes

Four fungal species were consistently isolated from symptomatic mango fruits across all surveyed regions: *Lasiodiplodia theobromae*, *Colletotrichum gloeosporioides*, *Alternaria alternata*, and *Curvularia spicifera* (Figure 1).

L. theobromae produced cylindrical, hyaline, septate paraphyses measuring up to 55 µm in length and 3-4 µm in width. Conidiogenous cells were holoblastic, cylindrical, and proliferated percurrently, forming 1-2 annellations or periclinal thickenings. Conidia were thick-walled, initially hyaline and aseptate, becoming dark brown and 1-septate post-discharge. They were subovoid to ellipsoid-ovoid (21-31 × 13-15.5 µm; length/width ratio = 1.9 ± 0.2), with a broadly rounded apex and truncate base.

A. alternata produced complex, branching conidial chains containing 5-60 conidia per chain. Mature conidia were narrowly ellipsoid to ovoid (10-30 × 5-12 µm), dull olive, with 3-7 transverse and 1-5 longitudinal septa, occasionally terminating in a short conical beak.

Conidiophores arose directly from the substrate, forming bushy heads composed of 4-8 short, 1-celled chains.

C. gloeosporioides developed acervuli bearing orange conidial masses. Conidiophores were irregularly branched, with subcylindrical, hyaline, smooth conidiogenous cells (10-25 × 2.5-4 µm) exhibiting percurrent proliferation and inconspicuous collarettes. Conidia were hyaline, smooth, guttulate, subcylindrical with blunt ends (12-17 × 4.5-6 µm). Germinating conidia produced 1-2 germ tubes, which developed clavate, aseptate appressoria measuring 7.1-8.6 × 4.7-6.0 µm. The teleomorph was not observed in culture.

C. spicifera formed dark brown to black, velvety colonies. Conidiophores were straight or slightly curved, septate, and 20-50 µm long. Conidia were multicellular, ellipsoidal to cylindrical (20-40 µm long), darkly pigmented, and borne in chains, consistent with *C. spicifera*, although minor morphological variations may occur due to environmental or host factors.

Table 5. Georeferenced data of surveyed orchards and SER incidence and severity (%) in Lodhran district.

Sub Locations	Orchard Name	Latitude (N)	Longitude (E)	(%) Disease Incidence	(%) Disease Severity
Lodhran	Pervaiz Qureshi Farm, Pulali Basti	29.5839	71.6272	19	7
	Waheed Qureshi, Lodhran-Multan Road	29.5822	71.6167	15	5.2
	Ali Tareen Mango Farm	29.4142	71.3241	13	5.8
	Mahar mango farm	29.3337	71.4142	11	4.4
	Bhutta mango farm	29.3306	71.40032	16	7
Kehror Pakka	Iqbal Shah Mango Farm, Ameer Pur	29.6404	71.8528	16	5.2
	Meo Mango Farm, Dhanote	29.6490	71.7796	19	7
	Buland Shah Gillani Mango Farm	29.6701	71.7739	12	4
	Dopal Shaheed Wala Bagh	29.6490	71.7799	14	4.4
	Shahzad Mango Orchard	29.6402	71.7234	11	4.2
Dunyapur	Waraich Mango Orchard, Dunyapur	29.8807	71.6300	14	5.6
	Huzeifa mango farm	29.8800	71.6291	19	5.8
	Waseem mango farm	29.8850	71.6325	17	5
	Zaman mango farm	29.5250	71.3748	11	4.4
	Babul mango farm	29.8721	71.6240	13	5.4

Table 6. Georeferenced data of surveyed orchards and SER incidence and severity (%) in Khanewal district.

Sub Locations	Orchard Name	Latitude (N)	Longitude (E)	(%)Disease Incidence	(%) Disease Severity
Khanewal	Raval Mango Farm, Khanewal	30.2117	71.4526	12	4.2
	Thaeem Fruit Farm	30.2457	71.5718	14	4.8
	Asghar Fruit Farm	30.3221	71.9857	17	5
	Arif Khagga Fruit Farm	30.3234	71.9839	11	5.6
	Mahar Azhar Fruit Farm	30.2825	71.9465	12	4
Kabir Wala	Samza Fruit Farm, Kabir Wala	30.2320	71.4729	12	4
	Mian Mousa Gardazi Farm	30.3972	71.8286	17	5
	Pir Bilal Shah Mango Orchard	30.4022	71.8281	18	7
	Haqnawaz Puni Model Farm	30.4411	71.8239	13	3.8
	Ashiq Gill Mango Farm	30.4528	71.6669	18	6.2
Jahanian	Malik Nabi Khan Maitla 112/10R	30.0402	71.7988	12	4
	Raheem Shah Mango Farm 110/10R	30.0458	71.8514	11	4.2
	Akhtar Wahla Farm 102/10R	30.8443	71.9132	12	4
	Kashif Thaeem Mango 55/10R	30.0925	71.9641	10	4
	Naseem Maitla Mango Farm 104/10R	30.1062	71.8907	11	4.2
Mian Channu	Hassaan Chishti Mango Orchard	30.5507	72.4634	12	4.8
	Channab mango farm	30.5492	72.4571	16	6.6
	Ghazanfar mango farm	30.4364	72.3425	19	6.8
	Kashif Naveed Mango farm	30.4304	72.3444	12	4.4
	Jahanzaib dharal mango farm	30.4309	72.3419	16	6.6

Table 7. Georeferenced data of surveyed orchards and SER incidence and severity (%) in Muzaffar Garh district.

Sub Locations	Orchard Name	Latitude (N)	Longitude (E)	(%)Disease Incidence	(%) Disease Severity
Muzaffar Garh	Ahmed Bhutta Mango Farm Rohila Wali	30.0958	71.2161	19	7
	Sheikh Imran Mango Farm Rohila Wali	30.0732	71.2331	17	5.8
	Kaleem Abid Mango Farm Makhan Bela	30.4722	70.9677	20	4.6
	Sajjad Mango Farm Wasanday Aali	30.0446	71.2033	12	6.8
	Sheikh Nasir Mango Farm Rohilawali	30.0669	71.2092	16	6
Alipur	Asif Mango Orchard, Head Dammer Wala	29.6560	70.9455	15	5.8
	Saith Adrees Mango Farm Chandar Aali	30.0652	71.2120	12	3.2
	Saleem Mango Farm Head Nalka	29.5947	70.9705	10	4.2
	Karam Hussain Ghallo Head Road Khan	29.6560	70.9455	17	6
	Riaz Gabol Mango Farm Chok Parmit	29.6560	70.9245	19	6.2
Jatoi	Khalid Bukhari Mango Farm	29.6062	71.0076	16	6.8
	Ishfaq Ahmed Kalar Ali Mango Farm	29.6012	71.0042	13	4.2
	Jind Wada Kalar Aali Mango Farm	29.6062	71.0076	18	6.4
	Asif Mango Farm Shehr Sultan Road	29.6419	70.9452	15	6.4
	Sajjad Ahmed Ghalwan Mango Farm	29.6546	70.9481	16	4.2
Kot Addu	Imran Hussain Mango Farm	29.7169	72.3711	17	6.4
	MALIK Abid Hanjra Deen Panah	30.4722	70.9677	15	6
	Mubarak Khan Mango Farm	30.4598	70.9528	13	4.6
	Ch. Yosuf Mango Farm	30.4713	70.9445	11	5.14
	Malik Asif Mango Orchard	30.4214	70.9234	11	4.6

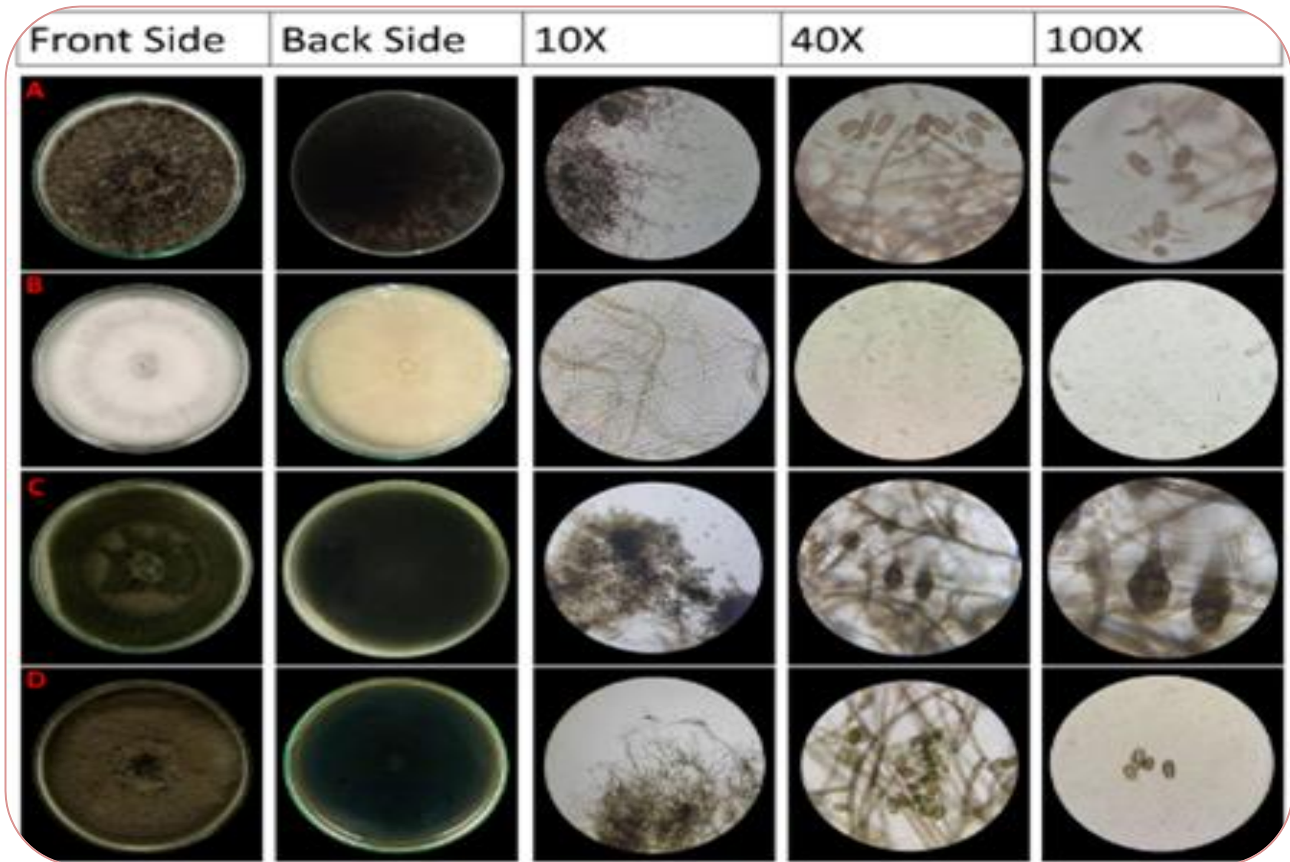

Figure 1. Morphological characteristics of fungal pathogens associated with SER of mango. Colony morphology (front and reverse views on PDA) and microscopic features observed at 10×, 40×, and 100× magnification of (A) *L. theobromae*, (B) *C. gloeosporioides*, (C) *A. alternata*, and (D) *C. spicifera*. Each row represents a single pathogen, showing colony appearance and diagnostic conidial structures.

Pathogenicity of fungi isolated from SER–affected mangoes

All fungal isolates induced typical stem-end rot symptoms on inoculated mango fruits, characterized by purplish discoloration near the peduncle that progressed to black necrotic lesions. Lesion size increased over the incubation period shown in (Figure 2), with *L. theobromae* producing the largest lesions (74.6 mm), followed by *C. spicifera* (54.6 mm), *C. gloeosporioides* (44.5 mm), and *A. alternata* (33.2 mm). No symptoms were observed in the control fruits. Pathogens re-isolated from the infected fruits were morphologically identical to the original cultures, thereby fulfilling Koch's postulates.

DNA extraction of fungal isolates

Genomic DNA was extracted from pathogenic fungal isolates using the CTAB method and assessed by electrophoresis on 1% agarose gel (Figure 3), which confirmed the presence of high-molecular-weight DNA in all samples.

PCR

The internal transcribed spacer (ITS) region was amplified using the universal primers ITS4 and ITS5. PCR products produced a single band of approximately 500 bp (Figure 4) upon agarose gel electrophoresis, indicating successful amplification.

Nucleotide sequencing

High-quality PCR amplicons were subjected to Sanger sequencing at Macrogen, Korea. The obtained ITS sequences were register in NCBI and assigned Accession numbers shown in (Table 8) and subsequently used to infer phylogenetic relationships among the isolates using maximum-likelihood and neighbor-joining analyses, thereby elucidating their taxonomic positions and evolutionary relationships.

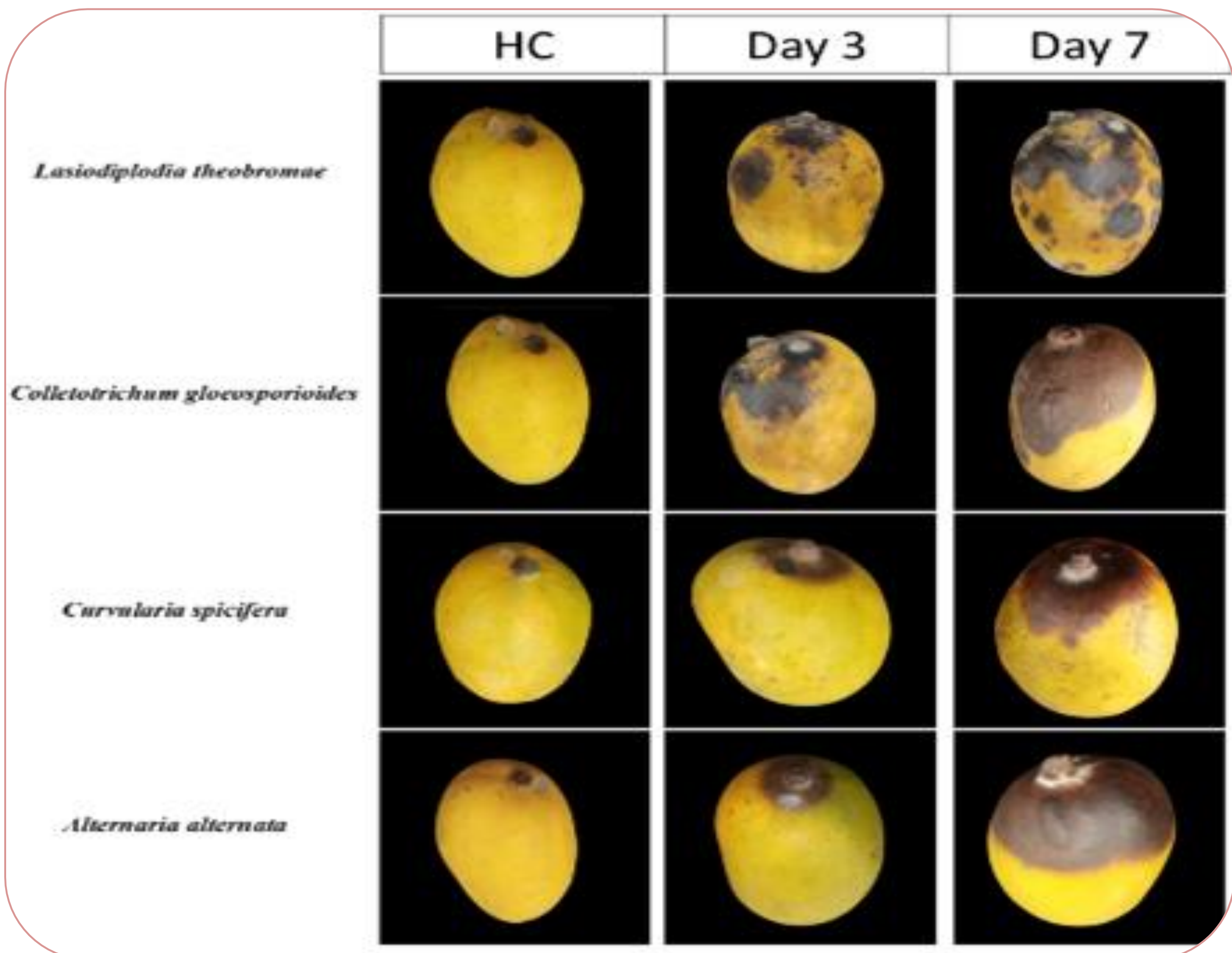

Figure 2. Pathogenicity of SER–associated fungi on mango fruits at the 3rd and 7th days post-inoculation. HC denotes the healthy control treated with sterile PDA.

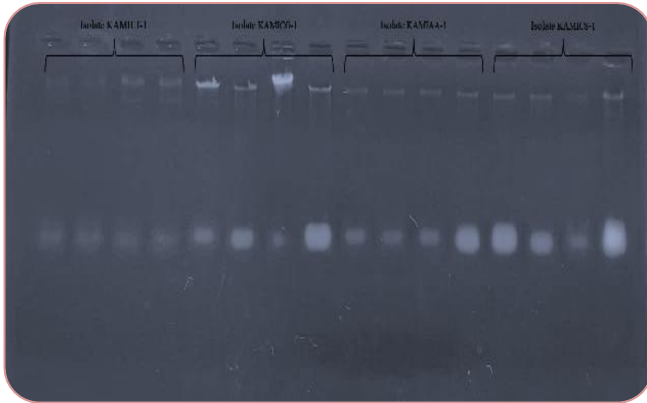

Figure 3. DNA bands of SER-associated fungal isolates.

Figure 4. PCR amplification of the ITS region from SER-associated fungal isolates using ITS1/ITS4 primers, showing a consistent ~550 bp amplicon in all isolates, confirming successful amplification.

Table 8. Molecular identification of SER-associated fungal isolates based on ITS sequence similarity with reference strains in GenBank.

Fungal pathogen	Isolate name	Accession number
L. theobromae	KAMILT-1	PQ608554
C. gloeosporioides	KAMICG-1	PQ608552
A. alterneta	KAMIAA-1	PQ607734
C. specifera	KAMICS-1	PQ607736

In vitro evaluation of fungicides against SER pathogens

The efficacy of four systemic fungicides viz., fludioxonil, azoxystrobin, difenoconazole, and fluoxastrobin was assessed against *L. theobromae*, *C. gloeosporioides*, *A. alternata*, and *C. spicifera* at concentrations ranging from 500 to 2000 ppm (Figure 5). Fludioxonil exhibited the highest inhibition of *L. theobromae*, with mycelial growth suppression increasing from 84.6% at 500 ppm to 91.9% at 2000 ppm. Difenoconazole (63.3-89.9%) and azoxystrobin (63.0-87.0%) demonstrated moderate to high efficacy, whereas fluoxastrobin was comparatively less effective (55.9-86.3%). These results highlight species-specific sensitivity and emphasize the importance of dose optimization for effective management of stem-end rot (Aulicky et al., 2025).

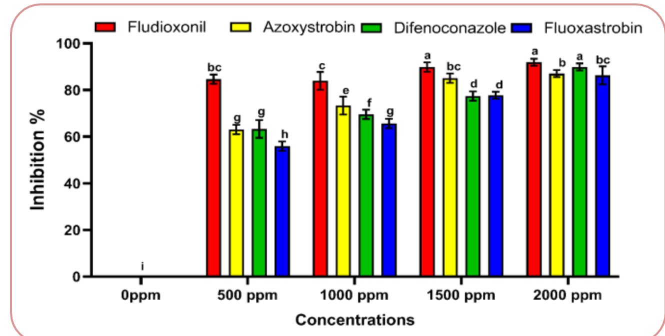

Figure 5. *In vitro* mycelial growth inhibition (%) of *L. theobromae* by fludioxonil, azoxystrobin, difenoconazole, and fluoxastrobin at concentrations ranging from 500 to 2000 ppm. Values represent the mean of replicates. Different letters above the columns indicate significant differences among treatments at p = 0.05, as determined by Tukey's HSD test. Error bars represent the standard error (SE) of the mean.

Fludioxonil exhibited the strongest inhibitory effect against *C. gloeosporioides*, with mycelial growth suppression increasing from 86.9% at 500 ppm to 94.4% at 2000 ppm, indicating a clear dose-dependent response (Figure 6). Azoxystrobin and difenoconazole demonstrated moderate to high efficacy, ranging from 62.2% to 92.3% and 49.3% to 90.7%, respectively, whereas fluoxastrobin was the least effective, showing 38.9% to 90.3% inhibition. Nevertheless, all fungicides achieved comparable levels of inhibition at the highest concentration tested.

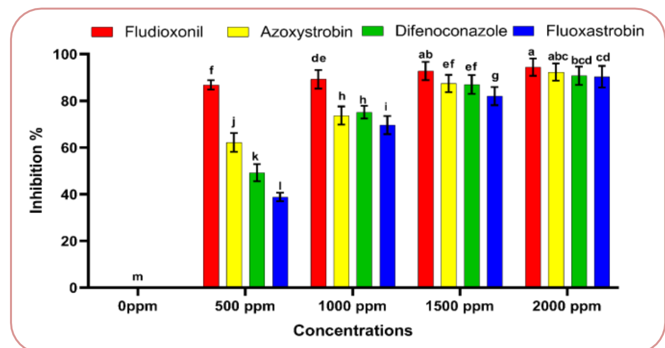

Figure 6. Mycelial growth inhibition (%) of *C. gloeosporioides* treated with fludioxonil, azoxystrobin, difenoconazole, and fluoxastrobin at 500–2000 ppm. Values represent the means of replicates. Different letters above the bars indicate significant differences among means at p = 0.05, as determined by Tukey's HSD test. Error bars represent the standard error (SE) of the mean.

Fludioxonil exhibited the strongest inhibition of *A. alternata*, with mycelial growth suppression increasing from 76.3% at 500 ppm to 90.7% at 2000 ppm. Azoxystrobin showed comparable efficacy (73.2-90.6%), whereas difenoconazole (35.9-90.9%) and fluoxastrobin (41.5-87.8%) demonstrated moderate to high activity only at higher concentrationsshown in (Figure 7). Overall, fungicide efficacy against *A. alternata* followed the order: fludioxonil > azoxystrobin > difenoconazole > fluoxastrobin.

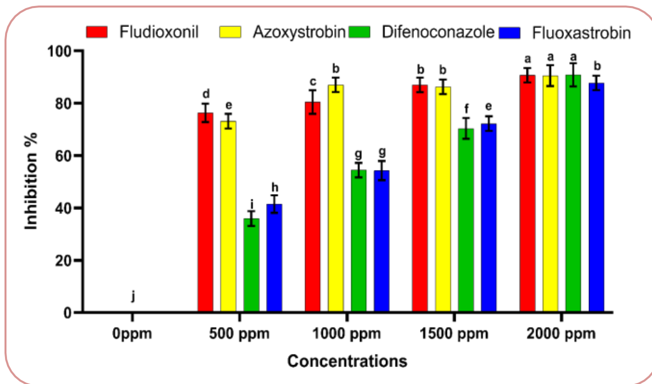

Figure 7. *In vitro* mycelial growth inhibition (%) of *A. alternata* by fludioxonil, azoxystrobin, difenoconazole, and fluoxastrobin at 500-2000 ppm. Values represent the means of replicates. Different letters above the bars indicate significant differences among means at p = 0.05, as determined by Tukey's HSD test. Error bars represent the standard error (SE) of the mean.

Fludioxonil was the most effective fungicide against *C. spicifera*, with mycelial growth inhibition increasing from 42.2% at 500 ppm to 90.2% at 2000 ppm (Figure 8). Azoxystrobin (37.8-87.4%) and difenoconazole (35.4-90.6%) also exhibited strong dose-dependent activity, whereas fluoxastrobin showed the lowest efficacy (41.5-87.7%). These findings underscore the potential of fludioxonil for managing *C. spicifera* in mango production.

Field efficacy of fungicides against naturally occurring SER in mango

Field trials were conducted in a commercial mango orchard using a randomized complete block design with five replicates to evaluate the efficacy of fludioxonil, azoxystrobin, difenoconazole, and fluoxastrobin at 500-2000 ppm against naturally occurring SER infection shown in (Figure 9). Untreated controls showed high disease incidence (71.3%) and severity (76%) (Figure 10). All fungicides significantly reduced both parameters, with fludioxonil demonstrating the highest efficacy. At 2000 ppm, fludioxonil reduced disease incidence and severity to 9.2% and 6%, representing 87.1% and 92.1% reductions, respectively. Even at 500 ppm, it achieved 60.2% and 65.8% reductions, indicating strong dose-dependent activity. Azoxystrobin (16.7% incidence, 14% severity) and difenoconazole (19.5%, 16%) provided substantial but lower control at 2000 ppm, while fluoxastrobin was the least effective (23.9%, 26%). These findings confirm fludioxonil as the most effective fungicide for SER management under field conditions, highlighting its potential for dose optimization to balance efficacy, cost, and environmental impact.

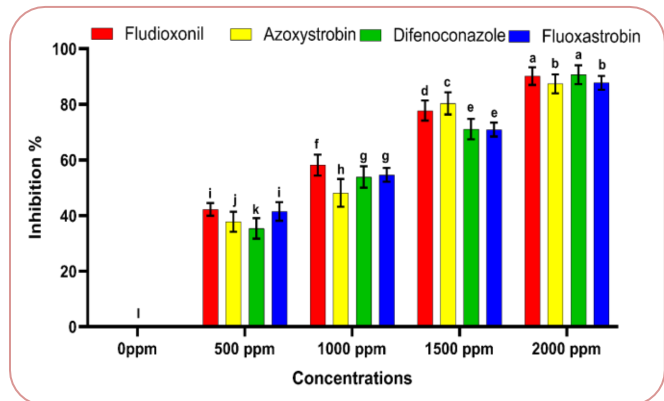

Figure 8. *In vitro* inhibition of *C. spicifera* mycelial growth (%) by fludioxonil, azoxystrobin, difenoconazole, and fluoxastrobin at concentrations of 500-2000 ppm. Values represent the means of replicates. Different letters above the bars indicate significant differences among means at p = 0.05, as determined by Tukey's HSD test. Error bars represent the standard error (SE) of the mean.

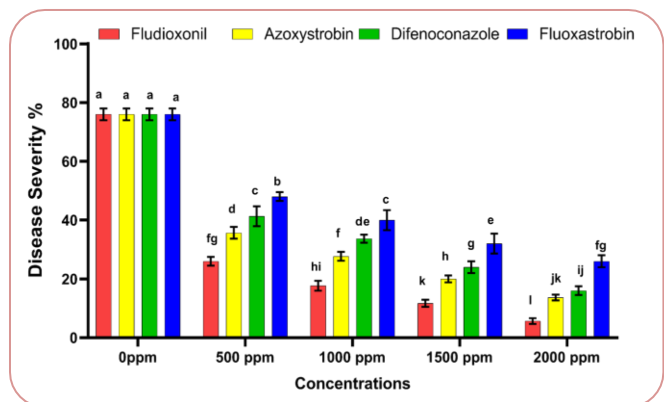

Figure 9. Effect of different fungicide concentrations on SER severity in mangoes under field conditions. Values represent the mean of replicates, and different letters above the bars indicate significant differences at p = 0.05 according to Tukey's HSD test. Error bars represent the standard error (SE) of the mean.

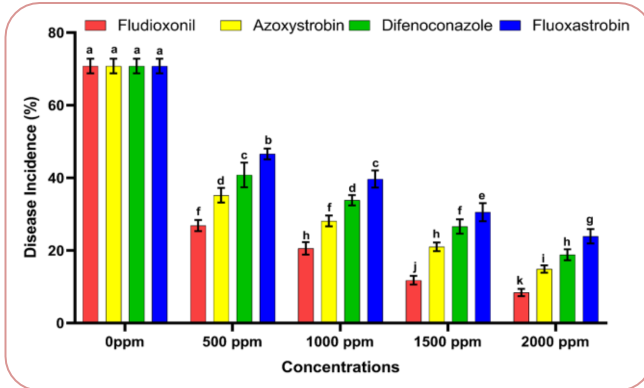

Figure 10. Effect of different fungicide concentrations on the incidence of SER in mangoes under field conditions. Values represent the mean of replicates. Different letters above the bars indicate significant differences among means at p = 0.05, as determined by Tukey's HSD test. Error bars represent the standard error (SE) of the mean.

Discussion

This study provides a systematic investigation of SER in mango across the principal production regions of Punjab, Pakistan, integrating field-level disease assessment, molecular characterization of pathogens, and fungicide evaluation. The combination of epidemiological, pathological, and chemical data offers a robust basis for disease management in an area where mango cultivation is both economically and culturally significant (Green et al., 2023; Naqvi et al., 2025a).

Field surveys across six districts (Bahawalpur, Rahim Yar Khan, Multan, Khanewal, Muzaffargarh, and Lodhran) revealed substantial regional variation in SER incidence, ranging from 33.3% in Yazman (Bahawalpur) to 83.3% in Muzaffargarh. High-incidence areas such as Muzaffargarh and Hasilpur (Bahawalpur) were characterized by older orchards of susceptible cultivars (e.g., Sindhri and Chaunsa) and suboptimal postharvest practices, including rough manual picking and inadequate orchard sanitation (Shah et al., 2025). These conditions favor pathogen entry and inoculum buildup, creating an environment conducive for SER development (Erayya et al., 2023). These findings are consistent with previous reports from Sindh, Pakistan, where SER prevalence averaged 65% (Khan et al., 2012; Baig et al., 2025), and from India, where high humidity and temperature fluctuations during fruit maturation were correlated with increased postharvest disease pressure (Kumar et al., 2015; Rangare et al., 2025). This regional heterogeneity highlights the need for site-specific management strategies rather than a uniform approach (Keith, 2020).

Four fungal pathogens viz., *L. theobromae*, *C. gloeosporioides*, *C. spicifera*, and *A. alternate,* were consistently isolated as pure cultures from symptomatic fruits across all districts (Kripa et al., 2024). Molecular identification using ITS sequence data confirmed the etiological role of these fungi in SER (Hussain et al., 2023). Although ITS provides limited resolution, phylogenetic analysis revealed clear clustering: Pakistani *L. theobromae* isolates (KAMILT-1) clustered with sequences from India and China, while *C. gloeosporioides* (KAMICG-1) grouped with isolates from China and Ethiopia (Ahmad et al., 2025), suggesting regional or intercontinental similarities likely driven by shared host varieties, agricultural trade, or environmental selection (Rosace et al., 2025). Nevertheless, ITS primarily indicates general genetic relatedness and may not capture fine-scale population structure or recent evolutionary events (Federici and Soddu, 2020; Naqvi et al., 2025b). The higher genetic divergence from Brazilian or Ecuadorian isolates likely reflects biogeographic isolation, though this remains speculative without broader genomic data (Muellner-Riehl and Rojas-Andres, 2022).

All four fungal species fulfilled Koch's postulates, confirming their pathogenicity in SER. *L. theobromae* was the most aggressive, producing necrotic lesions averaging 74.6 mm, significantly larger than those caused by *C. spicifera* (54.6 mm), *C. gloeosporioides* (44.5 mm), and *A. alternata* (33.2 mm) (Queirós et al., 2023). This gradient of virulence supports the classification of *L. theobromae* as the primary SER pathogen in tropical and subtropical mango systems, as reported in Sri Lanka, India, and Southeast Asia (Johnson et al., 2019; Gunamalai et al., 2023; Yeo et al., 2023). Their ability to establish latent infections during flowering and reactivate during ripening complicates control efforts that rely solely on decontamination (Nallathambi et al., 2020).

In vitro evaluation using the poisoned food technique indicated that fludioxonil was the most effective fungicide, inhibiting mycelial growth by over 90% at 2000 ppm (Chen et al., 2024). Its efficacy is attributed to its mode of action, which disrupts osmotic stress signaling in fungi by inducing uncontrolled glycerol accumulation and cell lysis, particularly effective against Botryosphaeriaceae like *L. theobromae* (Swart et al., 2006; Hayat et al., 2025). Azoxystrobin (a QoI fungicide) and difenoconazole (a DMI fungicide) exhibited intermediate efficacy, while fluoxastrobin was consistently the least effective, likely due to pre-existing

resistance or lower intrinsic activity against the isolates tested (Segalin, 2024).

Field trials confirmed fludioxonil's effectiveness, reducing SER incidence to 9.2% and severity to 6% at 2000 ppm (87.1% and 92.1% suppression compared with the untreated control, 71.3%). Even at 500 ppm, it reduced disease by over 60%, demonstrating a clear dose-response and potential for cost-effective application. These findings establish fludioxonil as a highly effective option for SER management in Pakistani mango orchards (Ahmad et al., 2025).

In summary, this study integrates epidemiology, pathogen diagnostics, and chemical control into a practical framework. Although future research should expand phylogenetic analysis and explore integrated management approaches, the present data provide producers with a science-based strategy to protect fruit quality, reduce postharvest losses, and enhance Pakistan's competitiveness in global mango markets (Naqvi et al., 2025a,b; Oltean et al., 2025).

Conclusion

Lasiodiplodia theobromae was confirmed as the dominant and most virulent pathogen causing SER in mango across Punjab, Pakistan. Isolates consistently produced the largest necrotic lesions (74.6 mm) in pathogenicity assays. Among the fungicides tested, fludioxonil was the most effective, achieving 87.1% reduction in incidence and 92.1% reduction in severity at 2000 ppm under field conditions, with significant activity observed even at 500 ppm. These results clearly demonstrate fludioxonil's strong efficacy against SER caused by *L. theobromae*, providing a validated chemical control option for affected mango orchards.

Authors' Contributions

MKA and MNA conceived and designed the study, performed data analysis, and drafted the original manuscript. MKA conducted field surveys, collected diseased samples, and carried out the isolation and identification of fungal pathogens. MTS and AM assisted with fungal identification and provided critical guidance, constructive feedback, and valuable suggestions to improve the content and clarity of the manuscript. All authors reviewed and approved the final version of the manuscript and agreed to be accountable for all aspects of the work, ensuring accuracy and integrity in reporting.

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Conflict of Interest

The authors declare no conflict of interest.

Sustainable Development Goals Targeted

SDG 2: Zero Hunger

SDG 9: Industry, Innovation, and Infrastructure

SDG 12: Responsible Consumption and Production

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