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Epidemiology, pathogenicity and sustainable management of *Fusarium oxysporum* causing leaf spot disease in Banana: Insights from Sindh Province, Pakistan

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Abstract

Banana leaf spot disease, caused by *Fusarium oxysporum*, poses a significant threat to banana cultivation. This study was conducted in Matiari and Tando Allahyar districts revealed notable regional differences in disease prevalence. In Matiari, the area of Tajpur exhibited highest incidence (45%), compared to Soomra Mori (33.5%) and Shahpur (24%). Similarly, in Tando Aallahyar, Nasarpur recorded prevalence of (35%), whereas Peer Kathi and Rashidabad showed lower incidence of (25% and 17%), respectively. Isolation and identification of the pathogen confirmed *F. oxysporum* as dominant causal agent, with higher prevalence observed in Matiari (64.7%) compared to Tando Allahyar (35.3%). Pathogenicity tests conducted through root-dipping and injecting methods demonstrated significant disease induction, root-dipping method resulting in highest disease incidence (16.5%) compared to 10.5% in injecting method. Root-dipping method also consistently showed the maximum number of disease spots (11.5%), followed by injecting method with (9%). Plant growth was significantly impacted by infection. Control plants displayed (24.5 cm in height), followed by plants inoculated via injecting method at 19.20 cm, while root-dipping method led to most severe stunting with (16.5 cm) in height. Efficacy of botanical extracts at different concentrations (10%, 20%, 30%, and 40%) against *F. oxysporum* was evaluated. Neem extract exhibited minimum fungal growth (6.5 mm at 40%) concentration. In comparison, Ginger and garlic extracts showed growths of (18.5 mm and 27.5 mm), respectively, while maximum fungal growth up to (98 mm) in control. Similarly, fungicides at various concentrations (50, 100, 150, and 200 ppm), minimum linear colony growth under Topsin-M was observed (3.4 mm at 200 ppm) Curzate and Aliette recorded higher linear growths of (8.5 mm and 17.5) mm, with maximum growth noted at (98 mm) in control. These findings offer valuable insights into developing integrated management strategies to banana leaf spot disease.

Keywords: Banana, Leaf spot, Incidence, *Fusarium oxysporum,* Pathogenicity, Management, Botanical Extracts, Fungicides

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Introduction

Banana (Musa spp.) is an important fruit crop globally and serves as a staple food and economic resource for millions of people (Zafarullah et al., 2021). Bananas are a vital source of income and nutrition for farmers in Sindh, Pakistan. The crop plays a pivotal role in ensuring food security and supporting the agricultural economy of the region (Rajput et al. 2015; Shah et al. 2022; Waseem et. al., 2022). Pakistan, particularly the Sindh province, is a significant contributor to banana cultivation, with a conductive climate that supports year-round production. In Sindh, several banana varieties are cultivated, including Basrai, Williams, Grand Naine, and Sindhri being prominent in the region. (Cannon et.al., 2022). However, banana cultivation faces several challenges, including fungal diseases, which result in substantial yield and quality losses. Among these, leaf spot disease caused by *F. oxysporum* is a prominent threat. This disease compromises photosynthetic efficiency, reduces fruit yield, and impacts plant health overall. The productivity of bananas is severely hampered by fungal pathogens, with leaf spot disease caused by *F. oxysporum* being a key concern (Dita et al., 2018).

This soil-borne fungal pathogen produces spores that can persist in soil and plant debris for extended periods, facilitating disease recurrence and spread (Ullah et al., 2023). Initial symptoms of the disease appear as small, water-soaked spots on leaves that enlarge, turning brown to dark brown with yellow halos. Severe infections result in coalesced lesions, leading to significant leaf blight. Affected leaves dry out, reducing photosynthetic activity and plant vigor. The fungus survives in soil and plant debris as chlamydospores and spreads through contaminated soil, water, and infected planting materials. Under favorable conditions, such as a warm and humid climate, the spores germinate and infect the leaf tissues. The fungus then invades vascular tissues, disrupting water and nutrient transport, which exacerbates disease symptoms and leads to more spores that facilitate further spread and recurrence of the disease (Dita et al., 2018).

Environmental factors such as temperature and moisture are crucial for chlamydospore germination, significantly influencing disease incidence. For instance, optimal growth conditions for *F. oxysporum* typically range around 25–29 °C, with the pathogen's activity greatly diminishing outside of this temperature range (Olivares et al., 2021). Banana leaf spot disease, primarily caused by the fungal pathogen *F. oxysporum*, poses a significant threat to banana cultivation in Sindh, particularly a survey of the banana-infected field was conducted in various regions of Sindh, Pakistan; Recorded disease incidence showed that Thatta was severely affected by leaf spot up to (46.66%), followed by Badin (28.88%) and minimum disease incidence was recorded in Hyderabad (24.44%) (Khaskheli et al., 2019; Irfanullah et al., 2021; Ahmed et al., 2021; Jatoi et al., 2024).

Effective management of this disease is crucial to sustaining banana yields, reducing economic losses, and ensuring the livelihoods of farming communities in the region. Such alarming figures necessitate urgent management strategies to mitigate the effects of *F. oxysporum* on banana crops. One promising avenue for disease management lies in the utilization of botanical extracts. Research has demonstrated that various plant-derived solutions can inhibit the growth of *F. oxysporum* effectively. For instance, aqueous leaf extracts of certain medicinal plants, including neem, have shown considerable effectiveness in controlling disease development in bananas (Thangavelu et al., 2013; Mangi et al., 2019; Mangi et al., 2021; Khan et al., 2021; Kakar et al., 2025). The application of these extracts not only reduces the incidence of leaf spot disease but also offers a sustainable alternative to synthetic chemicals, aligning with integrated management practices (Paramalingam et al., 2023). Complementing botanical interventions, the use of fungicides has been evaluated under laboratory conditions to determine their efficacy against *F. oxysporum*. Notably, studies have indicated that multi-site fungicides exhibit better control over fungal growth compared to single-site fungicides. These multi-site fungicides have multiple mechanisms of action, thereby reducing the possibility of pathogen resistance (Iqbal et al., 2019; Inayat et al., 2024; Jatoi et al., 2024; Kakar et al., 2025). Laboratory trials have revealed that certain fungicides, including captan and lipophilic cations, effectively suppress the growth of *F. oxysporum*, indicating their potential as viable treatments in managing banana leaf spot disease (Chanu et al., 2019; Cannon et al., 2022)

Materials and Methods

The research work was carried out in the Department of Plant Pathology, Faculty of Crop Protection, Sindh Agriculture University Tandojam.

Study area and sampling

The study was conducted in two major banana-growing districts of Sindh, Pakistan: Matiari and Tando Allahyar. Multiple locations within each district were selected based on disease prevalence. Matiari District locations Tajpur, Soomra Mori, and Shahpur and Tando Allahyar District locations Nasarpur, Peer Kathi, and Rashidabad. The number of infected and healthy leaves was counted to calculate the percent of disease incidence (PDI) by using the following standard formula:

$$\text{Percent Disease Incidence (PDI)} = \frac{Number\ of\ Infected\ Leaves}{Number\ of\ Observed\ Leaves} \times 100$$

Sampling

Diseased banana plants exhibiting characteristic symptoms of leaf spots were randomly sampled from the selected locations. A total of 60 leaf samples (10 from each location of both districts) were collected in sterilized bags and transported to the laboratory for pathogen isolation.

Isolation and identification of pathogens

The isolation of fungal pathogens was performed following standard protocol: Sample Processing, infected leaf was cut into 5-mm sections, surface sterilized using 1% sodium hypochlorite for 2 minutes, rinsed thrice in sterile distilled water, and dried on sterilized filter paper. Plating, sterilized leaf sections were plated on Potato Dextrose Agar (PDA) supplemented with streptomycin sulfate (0.05 g/L) to inhibit bacterial growth. Plates were incubated at 25 ± 2°C for 5–7 days. After the incubation period, data regarding the frequency percentage of targeted fungal colony growth were observed.

$$\text{Frequency percent} = \frac{No.of\ Tissues\ Plated\ Showing\ Targeted\ Fungal\ Colony}{Total\ No.of\ Fungal\ Colonies\ on\ Plated\ Tissue} \times 100$$

Pathogen purification

Fungal colonies were purified through small amounts of inoculum from freshly grown mycelial margins. The fungal culture inoculum was harvested using an isolation needle on the surface of one-day-old potato dextrose agar (PDA) medium. The petri plates were incubated at 28 ± 2 °C.

Morphological identification

Pathogens were identified based on colony morphology, spore characteristics, and microscopic observations using a compound microscope. *F. oxysporum* were identified as the primary pathogens.

Pathogenicity test

Preparation of inoculum

A conidial suspension of *F. oxysporum* was prepared by culturing the fungus on PDA for 7 days. The suspension was adjusted to 1 × 10^6 spores/mL using a hemocytometer. The pathogenicity of *F. oxysporum* was tested on young banana seedlings (5–6 leaf stage) under controlled conditions in (Fig. 1), two inoculation methods were employed:

Fig. 1 Pathogenicity test of leaf spot disease causing fungus *Fusarium oxysporum* on banana seedlings was conducted at seedling stage through root-dipping and injecting inoculation methods.

Root-dipping method

Roots of seedlings were immersed in the conidial suspension for 30 minutes before transplanting into sterilized soil pots (Jatoi et al., 2024).

Injecting method

A 2-mL conidial suspension was injected into the base of each seedling stem by using a sterile syringe. The data was recorded on plant disease incidence, plant height and number of leaf spots, before inoculation and after inoculation every 10, 20, and 30 days to evaluate the virulence of the pathogen. Uninoculated seedlings served as controls. Seedlings in the control were treated with sterile distilled water (Qambrani et al., 2022).

Evaluation of botanical extracts and fungicides

The antifungal efficacy of botanical extracts and synthetic fungicides against *F. oxysporum* was assessed using a poisoned food technique on PDA.

Botanical extracts

Extracts of neem (Azadirachta indica), ginger (Zingiber officinale), and garlic (Allium sativum) were prepared by blending fresh plant material with sterile distilled water (1:1 w/v). The extracts were filtered through muslin cloth and sterilized using a 0.22-µm membrane filter. PDA was amended with botanical extracts at concentrations of 10%, 20%, 30%, and 40% (v/v).

Fungicides

Synthetic fungicides, including Topsin-M (thiophanate-methyl), Curzate (cymoxanil), and Aliette (fosetyl-Al), were tested at concentrations of 50, 100, 150, and 200 ppm. Fungicides were dissolved in sterile water and incorporated into PDA.

Inoculation

A 5-mm fungal disc from a 7-day-old culture of *F. oxysporum* was placed at the center of each plate. Plates were incubated at 25 ± 2°C for 7 days, and radial colony growth was measured. Control: PDA plates without botanical extracts and fungicides served as controls. Both botanical extracts and fungicides percent inhibition of radial mycelial growth over the control was calculated using the following formula:

$$I = \frac{(C - T)}{C} \times 100$$

Where, I = Percent inhibition, C = Radial colony growth in control, T = Radial colony growth

Data analysis

The data on disease incidence were analyzed using a one-way ANOVA, the pathogenicity experiments were conducted in a completely randomized design (CRD) with three replicates per treatment. Disease incidence, plant height, number of leaf spots, and colony growth data were subjected to analysis of variance three-way ANOVA. The isolation of fungi was examined using a two-way ANOVA. One-way ANOVA was applied to analyze the data on botanical extracts and fungicide concentrations. Mean separations were performed using Tukey's Honestly Significant Difference (HSD) test after significant ANOVA results. Data were analyzed with Statistix

8.1, and all figures were generated using OriginPro 2024 software.

Results

Disease incidence

The leaf spot disease of banana shows significant variations in incidence across different locations in the Matiari district (Fig. 2), indicating distinct environmental factors affecting the disease's spread. A statistical analysis revealed a strong association between disease incidence and specific locales ($F = 10.13$, $df = 2, 29$; $P < 0.000$). Within the Matiari district, Tajpur (mean = 45, SE = 1.47) exhibited the highest disease incidence, followed by Soomra Mori (mean = 33.5, SE = 1.04), and Shahpur (mean = 24, SE = 0.74), demonstrating a clear gradient in disease prevalence. Similarly, in Tando Allahyar district (Fig. 3), significant variations in incidence were observed ($F = 35.63$, $df = 2, 29$; $P < 0.000$). The highest incidence in Tando Allahyar was recorded in Nasarpur (mean = 35, SE = 1.82), followed by Peer Kathi (mean = 25, SE = 0.73) and Rashidabad (mean = 17, SE = 0.69).

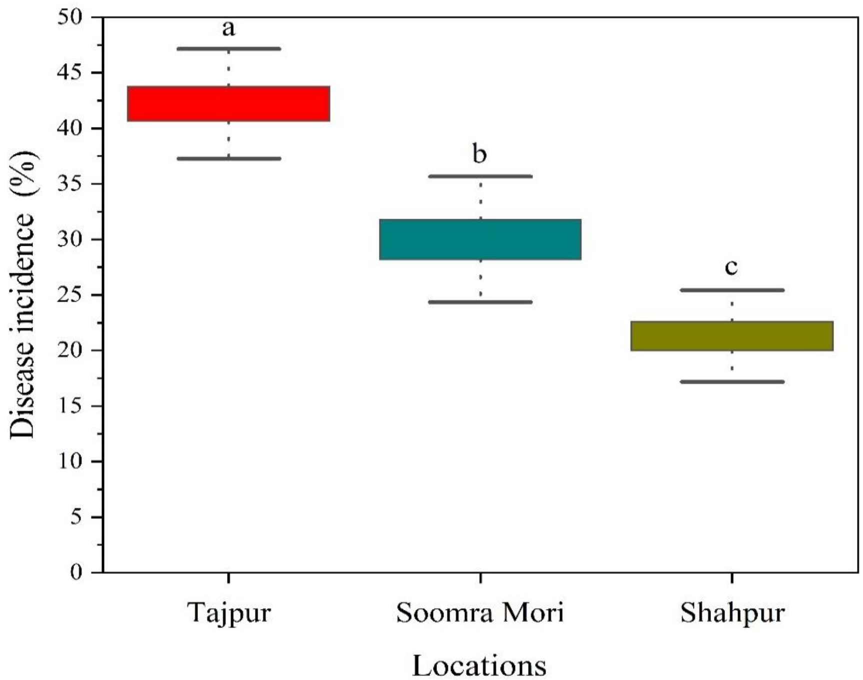

Fig. 2 Comparison of disease incidence on banana in different locations of district Matiari. Different colors of boxes represent the locations. The upper and lower bars indicate the maximum and minimum values, respectively. Different letters on bars indicate significant differences among treatments.

Isolation and identification of pathogen

Isolation and identification of the pathogen associated with banana leaf spot disease was successfully undertaken. Diseased banana leaf samples were collected from infected fields in both districts, leading to the isolation of *F. oxysporum.* The identification of this pathogen was confirmed through morphological and microscopic features, confirming its role in disease manifestation. Notably, *F. oxysporum* was found to be more prevalent in the Matiari district compared to Tando Allahyar, aligning with field observations of severe disease symptoms in the former region. Fungal diversity analysis highlighted *F. oxysporum* as the dominant pathogen in the Matiari district, showcasing a significantly higher frequency (64.7%) compared to Tando Allahyar (35.3%) in (Fig. 4).

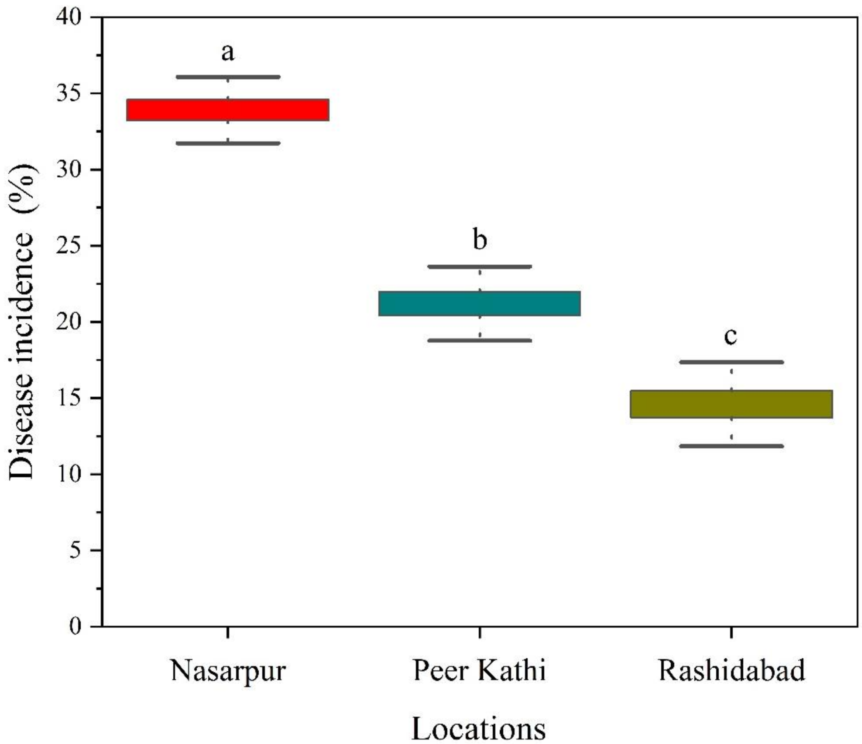

Fig. 3 Comparison of disease incidence on banana in different locations of district Tando allahyar. Different colors of boxes represent the locations. The upper and lower bars indicate the maximum and minimum values, respectively. Different letters on bars indicate significant differences among treatments.

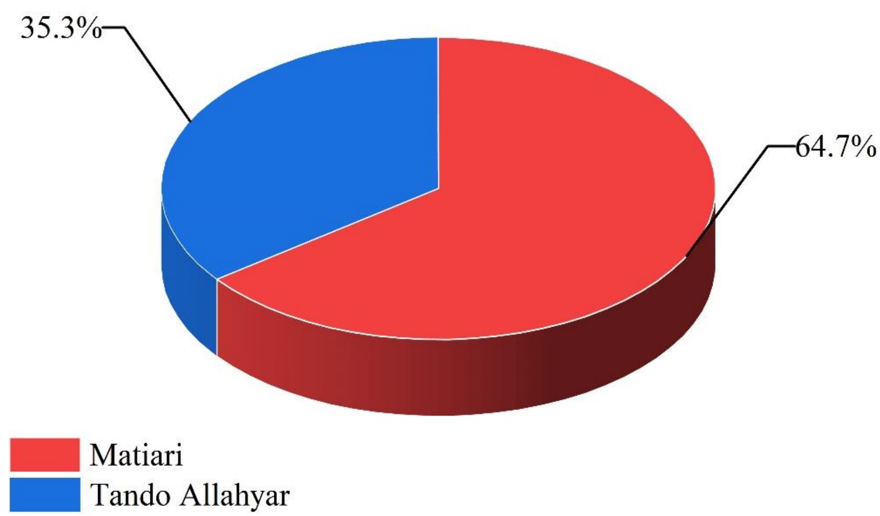

Fig. 4 District-wise percentage of leaf spot disease causing fungi *Fusarium oxysporum* of banana

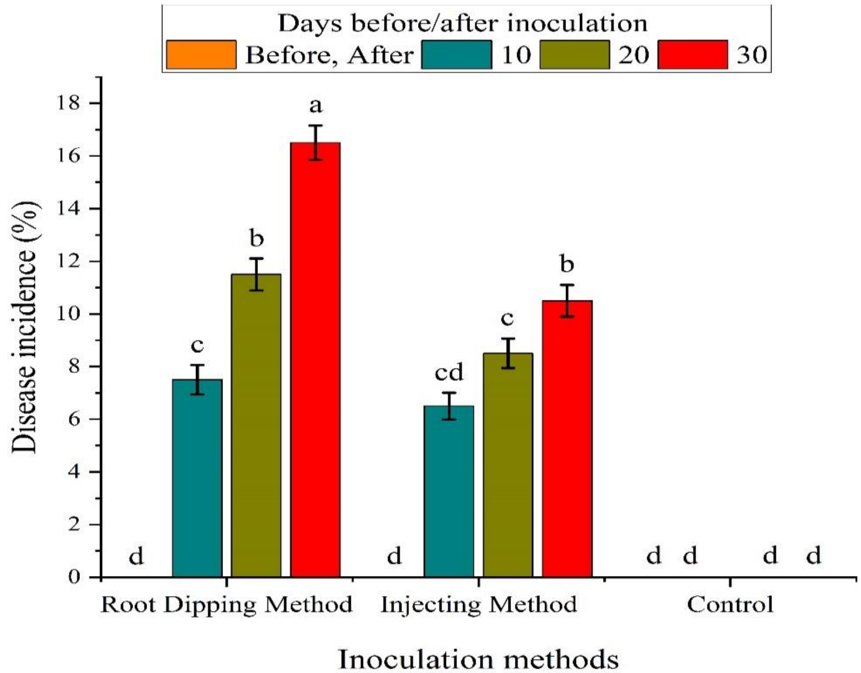

Fig. 5 Effect of leaf spot disease causing fungus *Fusarium oxysporum* on leaves (disease incidence percent) on banana seedlings (interaction between inoculation methods and days)

Pathogenicity test

The pathogenicity of *F. oxysporum* was assessed using two inoculation methods: the root-dipping method and the injecting method. Statistical analyses revealed that disease incidence was significantly associated with both inoculation methods and the days elapsed ($F = 13.77$, $df = 2, 8$; $P < 0.05$). Fig. 5 shows that root-dipping method caused the highest disease incidence (mean = 16.5, SE = 1.32), demonstrating its effectiveness in establishing infections. The injecting method also resulted in disease symptoms but at lower levels (mean = 10.5, SE = 0.73), while control plants remained free of disease symptoms, thereby confirming the pathogenicity of the isolated fungus. These results indicated significant associations with inoculation methods, days, and the number of disease spots on the plants ($F = 6.98$, $df = 2, 8$; $P < 0.05$). Fig. 6 shows that root-dipping method consistently yielded the maximum number of disease spots (mean = 11.5, SE = 0.73), followed by the injecting method (mean = 9.00, SE = 0.56). Control plants remained free from any disease symptoms. Moreover, the height of the plants was significantly affected by the infection process ($F = 1.93$, $df = 2, 11$; $P < 0.05$). Fig. 7 shows that control plants displayed the greatest growth (mean = 24.5, SE = 0.64), followed by plants inoculated using the root-dipping method (mean = 16.5, SE = 0.50), with the injecting method leading to the most severe stunting (mean = 19.20, SE = 0.57). These findings confirm the high virulence of *F. oxysporum* and support the use of the root-dipping method for effective pathogen inoculation studies.

Efficacy of botanical extracts and fungicides

Statistical analysis of the effect of botanical extracts on linear colony growth yielded significant results ($F = 94.9$, $df = 3, 129$; $P < 0.000$), evaluating the potential of these extracts to inhibit the growth of *F. oxysporum*. Among the tested extracts, the neem extract demonstrated the strongest antifungal activity (mean = 20.12, SE = 1.83), significantly inhibiting the linear growth of *F. oxysporum* (Fig. 8). In contrast, ginger (mean = 33.87, SE = 1.83) and garlic (mean = 42.37, SE = 1.83) extracts showed comparatively higher fungal growth in comparison to neem. The control group exhibited the most extensive fungal growth, reaching a mean of 98 mm (SE = 2.04). Under various concentrations (10%, 20%, 30%, and 40%), the linear colony growth under neem extract was measured at 37 mm, 23 mm, 14 mm, and 6.5 mm, respectively. Ginger extract resulted in measurements of 49 mm, 38.5 mm, 29.5 mm, and 18.5 mm, while garlic extract recorded maximum growth of 60 mm, 45.5 mm, 36.5 mm, and 27.5 mm at the respective concentrations. The control conditions yielded fungal growth up to 98 mm. Statistical analysis revealed significant differences in efficacy among botanical extracts at various concentrations. Additionally, the statistical analysis for the effect of fungicides on linear colony growth showed significant results ($F = 138$, $df = 3, 129$; $P < 0.000$). Fig. 9 shows that lowest growth was recorded with Topsin-M (mean = 15.12, SE = 1.80), followed by Curzate (mean = 23.25, SE = 1.80) and Aliette (mean = 34.34, SE = 1.80). The control group, conversely, recorded the highest growth (mean = 98, SE = 2.01). At various concentrations (50, 100, 150, and 200 ppm), the minimum linear colony growth under Topsin-M was observed at 26 mm, 13.5 mm, 6.6 mm, and 3.4 mm, respectively. Curzate measured 38 mm, 27.5 mm, 19 mm, and

8.5 mm. Maximum growth was noted under Aliette at 55.5 mm, 36.5 mm, 26.5 mm, and 17.5 mm at the respective concentrations. Again, statistical analysis exhibited significant differences among fungicides and their concentrations, underscoring their effectiveness in managing *F. oxysporum*.

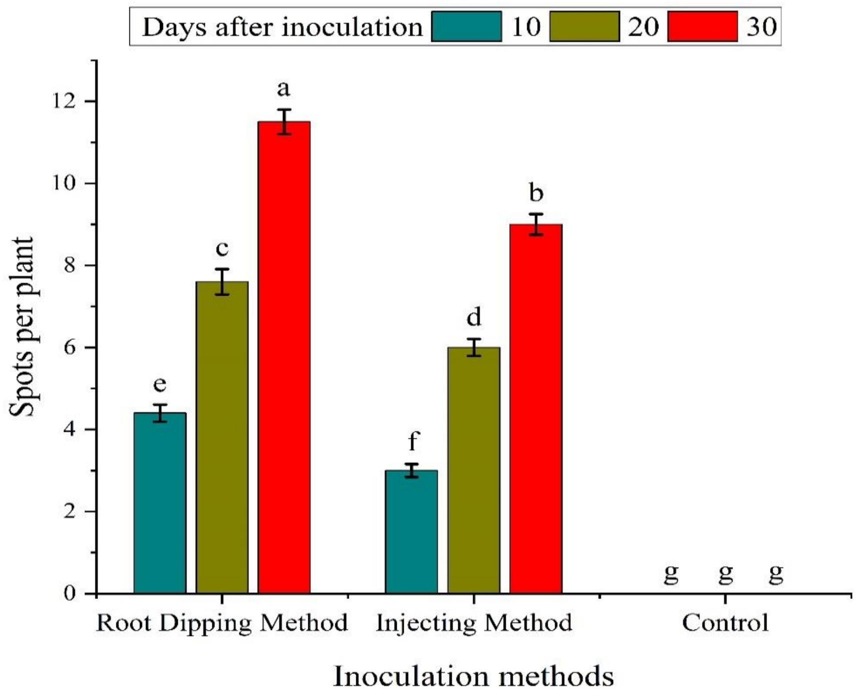

Fig. 6 Effect of necrotic spots caused by leaf spot disease causing fungus *Fusarium oxysporum* on banana seedlings (interaction between inoculation methods and days)

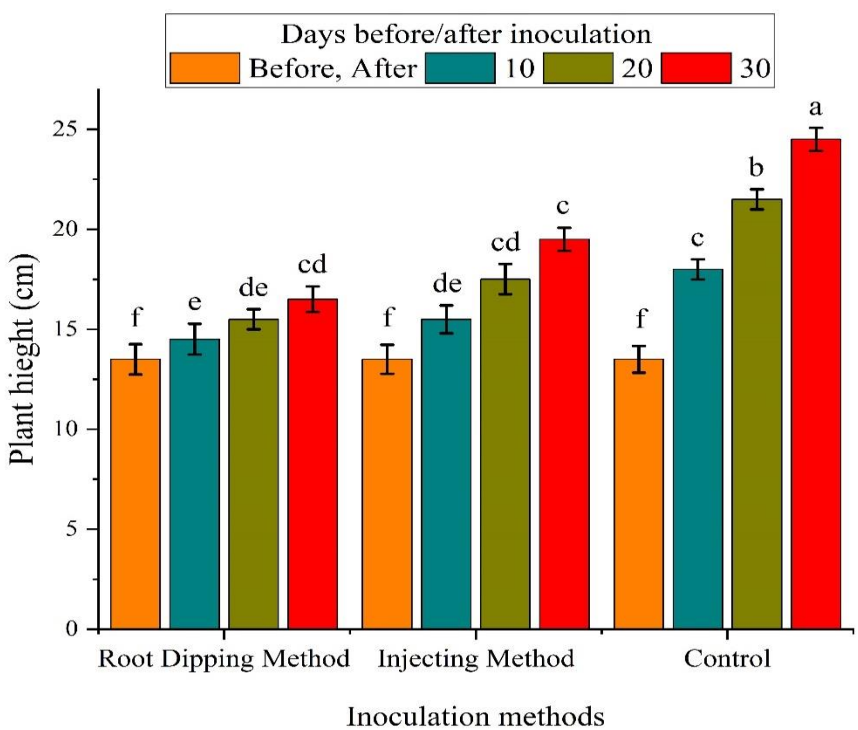

Fig. 7 Effect on plant height (cm) caused by leaf spot disease causing fungus *Fusarium oxysporum* on banana seedlings (interaction between inoculation methods and days)

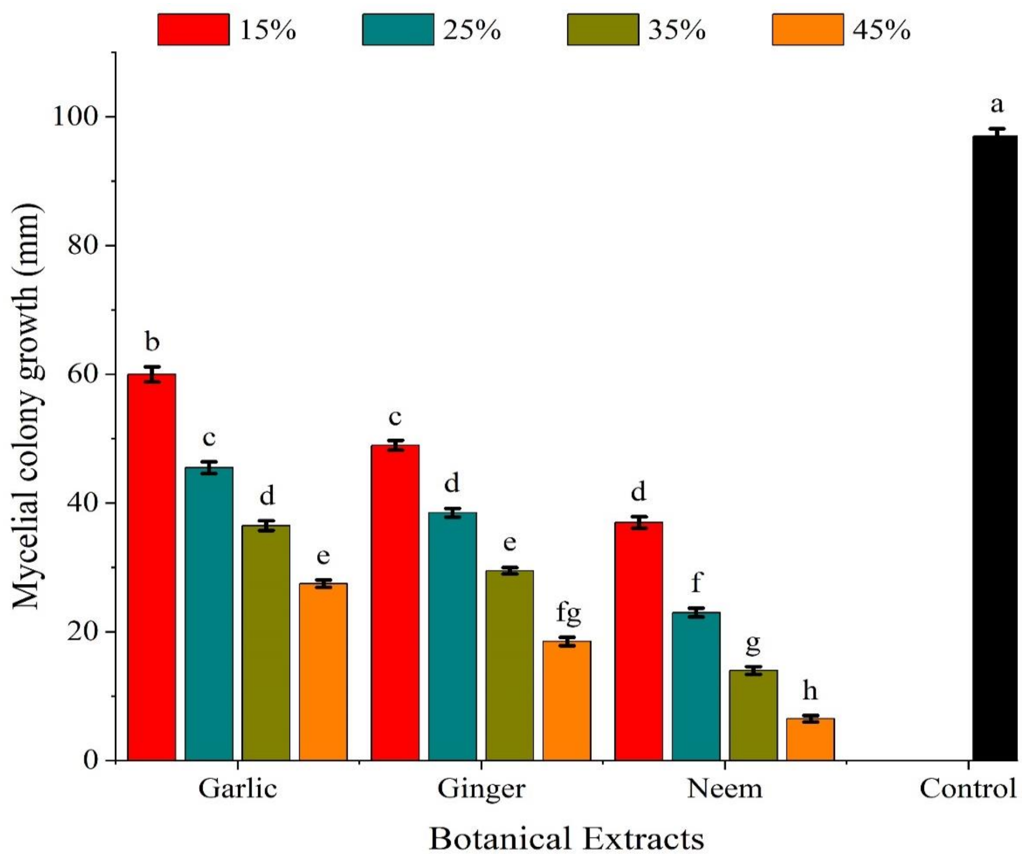

Fig. 8 Comparison of mycelial colony growth (mm) of *Fusarium oxysporum* fungus at different concentrations of botanical extracts, compared with control. Different colors of bars represent (concentration in %). Different letters on bars indicate significant differences among treatments.

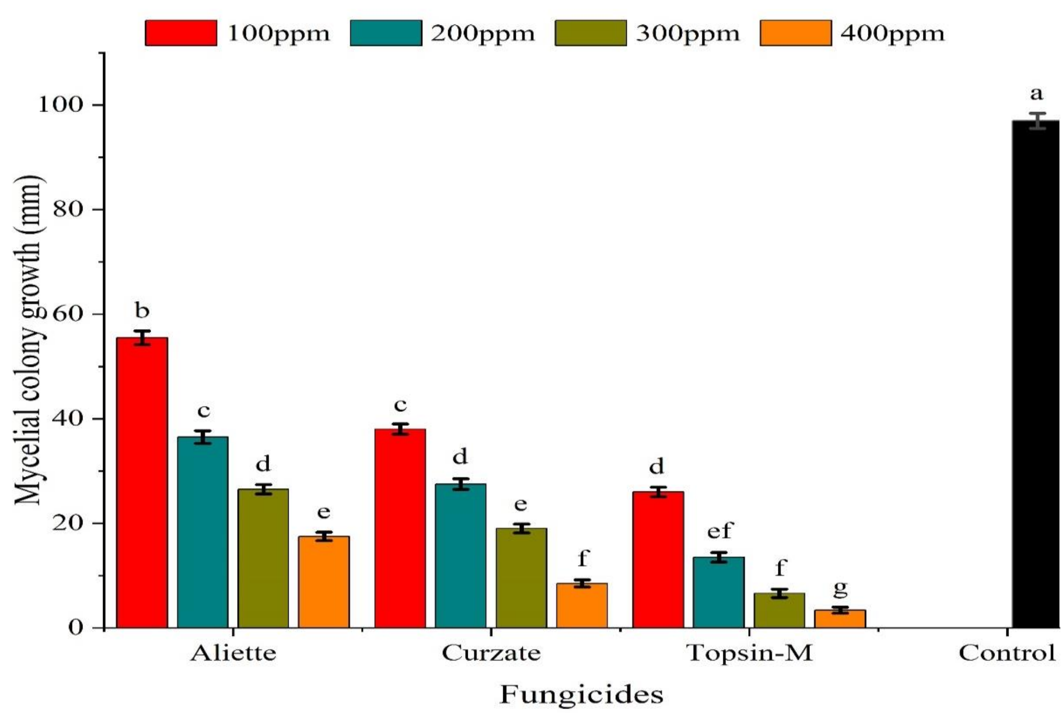

Fig. 9 Comparison of mycelial colony growth (mm) of *Fusarium oxysporum* fungus at different concentrations of fungicides, compared with control. Different colors of bars represent (concentration in ppm (part per million)). Different letters on bars indicate significant differences among treatments.

Discussion

The results of this study reveal significant geographic variations in the incidence of banana leaf spot disease within the Matiari and Tando Allahyar districts of Pakistan. Tajpur in the Matiari district had the highest incidence rate (mean = 45), which could be attributed to a combination of environmental factors, agronomic practices, and potentially the climatic conditions unique to this area. The significant statistical correlation ($P < 0.000$) between disease incidence and location highlights the necessity for localized studies to understand better the environmental drivers behind such variations. While similarly in Tando Allahyar, the disease incidence was also noted across locations, with Nasarpur showing the highest figures (mean = 35). The statistical significance ($F = 35.63$, $P < 0.000$) in both districts indicates a systematic pattern of disease spread likely influenced by agronomic factors such as crop rotation, planting density, and moisture levels, which have been observed to affect disease prevalence in agricultural contexts. Such findings are consistent with earlier research that has documented geographically distinct incidences of similar diseases (Khaskheli et al. 2019; Qambrani et al. 2019; Qambrani et al. 2022; Jatoi et al. 2024).

The identification of *F. oxysporum* as the causal agent of the leaf spot disease through both morphological and microscopic analysis confirms findings from various previous studies that have implicated *F. oxysporum* in banana diseases. The higher prevalence rate in the Matiari district (64.7%) compared to Tando Allahyar (35.3%) may be reflective of a more conducive environmental niche or perhaps higher pathogen inoculum levels in that region. The significant variance in fungal frequency underscores the pressing need for tailored management strategies to mitigate its spread effectively (Qambrani et al. 2019; Qambrani et al. 2022; Jatoi et al. 2024).

The assessment of pathogenicity between inoculation methodologies produced noteworthy insights. The root-dipping method (mean = 16.5) produced a notably higher incidence compared to the injecting method (mean = 10.5), reinforcing the concept that soil-borne pathogens like *F. oxysporum* are likely to establish more effectively through root exposure. Such findings underscore the importance of employing effective inoculation techniques in both experimental and field settings for better disease management practices. Moreover, the effect of plant growth on disease incidence is significant, illustrating the complex interplay between plant health and fungal infection. The diminished growth observed in infected plants, particularly in those subjected to the injecting method, emphasizes the detrimental impact of *F. oxysporum* on banana cultivation. The height of control plants being the greatest further validates the assertion that robust plant health is critical for resisting pathogen invasion (Qambrani et al. 2022; Jatoi et al. 2024).

Efficacy testing of botanical extracts yielded substantial results that advocate for their role in the management of *F. oxysporum.* Notably, neem extract exhibited the most substantial antifungal activity, significantly inhibiting the linear growth of the pathogen (mean = 20.12), which concurs with historical research affirming the plant's bioactive constituents' effectiveness against various fungal pathogens. Conversely, although garlic and ginger extracts showed some efficacy, they were less effective than neem, emphasizing the need for selecting potent natural remedies in managing plant diseases (Mangi et al., 2019; Mangi et al., 2021; Khan et al., 2021; Kakar et al., 2025). Similarly, the fungicides demonstrated efficacy in inhibiting fungal growth, with Topsin-M being the most effective (mean = 15.12) and significantly suppressing pathogen activity. This aligns with existing knowledge regarding fungicide use in agricultural practices where chemical control measures are deemed essential for managing soil-borne pathogens like *F. oxysporum.* The differences showcased among fungicides confirm the necessity for comparative studies to identify the most effective treatments for specific pathogens, which can inform integrated plant disease management strategies (Iqbal et al., 2019; Chanu et al., 2019; Cannon et al., 2022; Inayat et al., 2024; Jatoi et al., 2024; Kakar et al., 2025).

Conclusion and recommendations

The study highlights significant variations in banana leaf spot disease incidence across different locations, influenced by environmental factors. *F. oxysporum* was identified as the primary pathogen, with higher prevalence in the Matiari district. Pathogenicity tests confirmed its virulence, with the root-dipping method being the most effective for infection. Neem extract exhibited the strongest antifungal activity among botanical treatments, while Topsin-M proved to be the most effective fungicide. Statistical analyses validated significant differences in the efficacy of both botanical extracts and fungicides. These findings provide valuable insights for disease management strategies in Banana cultivation. To effectively manage banana leaf spot disease caused by *F. oxysporum*, it is essential to utilize neem extract due to its strong antifungal properties and consider the implementation of Topsin-M as part of a fungicide strategy. Additionally, regular monitoring of environmental factors and disease incidence across various locales can inform timely interventions and improve overall crop health. Integrating cultural practices aimed at reducing humidity and enhancing drainage will further assist in mitigating disease spread.

Conflict of Interest The authors declare that they have no conflict of interest.

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