Biological Forum
an International Journal 17(6): 38-52(2025)
ISSN No. (Print): 0975-1130
ISSN No. (Online): 2249-3239

Assessment of Fungicide Efficacy in Managing Pomegranate wilt Disease caused by *Ceratocystis fimbriata* and *Fusarium oxysporum*

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(Received: 19 March 2025; Revised: 27 April 2025; Accepted: 22 May 2025; Published online: 13 June 2025)
(Published by Research Trend)

ABSTRACT: Pomegranate wilt disease causes significant damage in orchards due to the complete drying and death of affected plants, resulting in considerable economic losses to growers. The disease is characterized by yellowing, wilting, and drying of foliage, and it spreads rapidly through soil and water posing a serious threat to nearby healthy plants. Two fungal pathogens, *Ceratocystis fimbriata* and *Fusarium oxysporum*, were identified to be associated with it based on morphological and cultural characteristics. Pathogenicity tests confirmed that *C. fimbriata* induced symptoms more rapidly than *F. oxysporum*, and co-inoculation of both pathogens led to accelerated disease development, suggesting a synergistic interaction. This study evaluated the efficacy of various fungicides under *in vitro*, pot, and field conditions and revealed that the treatment Propiconazole 25% EC and Carbendazim 12% +Mancozeb 63% WP exhibited the highest inhibition of mycelial growth of *C. fimbriata* and *F. oxysporum* under *in vitro* condition. The efficacy of these treatments was further validated through pot and field experiments, which revealed significant reductions in soil pathogen inoculum (cfu/g) and disease severity, demonstrating their potential for effective management of soil-borne pathogens under field conditions. Accurate pathogen identification is essential for effective management, which depends on early detection, timely fungicide application, improved soil drainage, and removal of infected plants. The difficulty in managing severely affected plants highlights the need for early intervention and integrated disease management strategies.

Keywords: Pomegranate wilt, Soil-borne pathogens, *Ceratocystis fimbriata*, *Fusarium oxysporum*, Fungicide efficacy.

INTRODUCTION

Pomegranate (*Punica granatum* L.) is a deciduous, multi-stemmed shrub or small tree reaching 5–8 m in height, cultivated for its fruits valued for both nutritional and therapeutic properties, being rich in carbohydrates and essential minerals such as calcium, iron, and sulfur (Palou and Del-Rio 2009). Arid and semi-arid regions with 500–1000 mm of annual rainfall, hot and dry summers, and mild winters are ideal for pomegranate cultivation, as the crop requires a warm, dry climate during the fruit ripening stage. The plant thrives well in medium-deep, loamy, well-drained soils with a pH of 7.5 and prefers temperature ranging from 23 to 32°C. It is extensively grown in Asia, the Middle East and the Mediterranean region with major producers including India, Iran, China, Turkey, Afghanistan, Egypt, and Spain. In India, pomegranate cultivation is primarily concentrated in Maharashtra, Gujarat, Karnataka, and Andhra Pradesh. In contrast, Himachal Pradesh accounts for a smaller share, with cultivation spread over 2,880 hectares and an annual production of 3,271 metric tons of fruit (Anonymous, 2021a; 2021b). Its cultivation has faced increasing challenges due to a combination of biotic and abiotic stresses affecting its aerial and root systems. Foliar pathogens mainly affect photosynthesis and diminish the fruit quality and market value, whereas soil-borne

pathogens pose a more serious threat by causing complete plant death. Wilt is a major soil-borne disease that causes yellowing and drying of pomegranate plant. This disease has been reported in several countries, including Armenia, North America, France (Ferrari and Pechenot 1974), Slovakia, Switzerland (Matasci and Gessler 1997), Italy (Panconesi, 1999), India (Somasekhara, 1999), China (Huang *et al*., 2003), and Pakistan (Alam *et al.,* 2017). In Himachal Pradesh, it has previously been documented in the districts of Bilaspur, Kullu, Mandi, and Hamirpur affecting pomegranate orchards (Khosla *et al.,* 2011).

This disease threatens the economic stability of farmers worldwide because of the drying of fruits, flowers, and leaves that causes complete reduction of yield from infested plants. Several pathogens have been identified to be associated with this disease, including *Ceratocystis fimbriata*, *Fusarium oxysporum*, *Fusarium solani*, *Rhizoctonia* spp., and *Meloidogyne incognita* (Sharma *et al.,* 2010). However, this study specifically focuses on assessment of fungicide efficacy for managing *C. fimbriata* and *F. oxysporum*. Studies related to *M. incognita* have been published separately and are therefore not discussed in detail in this publication (Mukesh *et al.,* 2024). *C. fimbriata* and *F. oxysporum* commonly enter through root injuries caused by intercultural operations. *C. fimbriata* invades xylem and phloem, secreting enzymes like cellulases and pectinases that degrade cell walls. *F. oxysporum* enters through root wounds and spreads within the xylem, where its hyphae and spores, along with host responses like tyloses and gum deposition, block water flow, leading to wilt symptoms development (Yadeta and Thomma 2013). *C. fimbriata* produces endoconidia, aleuroconidia, and chlamydospores, while *F. oxysporum* forms macroconidia, microconidia, and chlamydospores that enable both pathogens to persist in soil for long periods in the absence of a host (Nasution *et al.,* 2019; Haware *et al.,* 1996).

The persistence of soil-borne pathogens and their spread through irrigation water and infected plant material make their management particularly difficult. These diseases pose a serious threat to the sustainability and profitability of pomegranate orchards, especially in monoculture systems with inadequate disease control. Although biological control methods (bioagents and soil amendments) offer sustainable options when used preventively, they are generally ineffective against active infections under field conditions. In contrast, fungicides provide more reliable control of ongoing infections. Systemic fungicides are absorbed and translocated within the plant, offering internal protection, while non-systemic fungicides act externally through contact. Combination fungicides, which merge both properties, offer broader-spectrum defense. Fungicides act by inhibiting pathogen growth or interfering with microbial enzyme activity in the soil (Kenarova and Boteva 2023). However, excessive use, particularly of triazole-based compounds, may negatively impact beneficial soil microbes and enzymatic processes (Roman *et al.,* 2021). The effectiveness of fungicides against *C. fimbriata* and *F. oxysporum* depends on their mode of action, application timing, environmental conditions, and pathogen resistance. Field management remains challenging due to variable weather, soil characteristics, and evolving pathogen resistance. This study, therefore, evaluated the performance of systemic, non-systemic, and combination fungicides under *in vitro*, pot, and field conditions to identify the most effective treatments for reducing fungal load in wilt-infested pomegranate tree basin soil.

METHODS

The study was conducted at the department of Plant Pathology, Dr YS Parmer University of Horticulture and Forestry Nauni, Solan, Himachal Pradesh, India.

A. Isolation and identification of C. fimbriata and F. oxysporum

Infected root samples were chopped surface sterilized, and plated on Potato Dextrose Agar (PDA). Rhizospheric soil samples (100 g each) were collected from the basins of wilt-affected pomegranate trees at a depth of 10–25 cm and pooled to form a composite sample. Fungal populations were assessed using the serial dilution plate method. One gram of composite soil was suspended in 9 ml sterile distilled water (10^{-1} dilution), followed by serial dilutions. From each dilution, 0.1 ml was plated onto selective media. *C. fimbriata* was isolated on V8 juice agar (200 ml V8 juice, 3 g $CaCO_3$, 20 g agar per liter), and *F. oxysporum* on Malachite Green Agar (2.5 ppm) containing 15 g peptone, 1 g KH_2PO_4, 0.5 g $MgSO_4 \cdot 7H_2O$, 20 g agar, and 0.0025 g malachite green oxalate per liter (Jeschke *et al.,* 1990; Bragulat *et al.,* 2004). Plates were incubated at 25 °C and emerging colonies were purified on PDA medium. The morphological characteristics of *C. fimbriata* and *F. oxysporum* were examined by preparing slides from mature cultures and observing spore and conidial structures under a microscope at 40× magnification (Windels, 1991). Identification was carried out using a taxonomic key and confirmed by comparing measurements with descriptions from previous studies (Hunt, 1956; Leslie and Summerell 2006).

B. Pathogenicity test

Pathogenicity test was conducted on healthy pomegranate seedlings (cv. Kandhari Kabuli) grown in plastic pots under greenhouse conditions at the Department of Plant Pathology. *C. fimbriata* culture was mass-multiplied on wheat bran, and *F. oxysporum* on maize seed medium. For culture preparation, wheat and maize seeds were boiled until softened. Excess water was drained, and the seeds were mixed with sawdust (for wheat) or sand (for maize) in a 1:3 ratio (sawdust/sand: seed) to prevent clumping. The mixtures were sterilized using the tyndallization method with three consecutive days of heating at 100°C in an autoclave for 15 minutes at 5 psi pressure (Negi and Gautam 2013). After sterilization, the media were inoculated with actively growing both fungal cultures and incubated at 25°C for 30 days to allow for proliferation. Each pathogens inoculums was applied at a rate of 2 g/kg soil, totaling 10 g inoculum per pot,

placed near the seedling roots (Chaudhary *et al.*, 2017). To enhance infection, small cuts were made on the root surface. Pots were regularly irrigated to maintain optimal moisture. Disease development was monitored, and the number of days until first symptom appearance was recorded. Each pathogen was re-isolated from the infected roots of newly infested plants onto PDA and identified by comparing its cultural and morphological characteristics with those of the original isolates (Agrios, 2005).

C. In-vitro evaluation of various fungicides against mycelial growth of C. fimbriata and F. oxysporum

Various systemic fungicides (difenoconazole 25%EC, propiconazole 25% EC, thiophanate methyl 70% WP and fosetyl-Al 80% WP) were evaluated at 100,200 and 300ppm concentration and non systemic fungicides (chlorothalonil WP and copper oxychloride 50 WP) were tested at 250,500,1000ppm while the combi fungicide (metiram+pyraclostrobin 60 WG and carbendazim 12% +mancozeb 63% WP) were tested at 100,250, 500ppm concentration against mycelial growth of *C. fimbriata* and *F. oxysporum* using the poison food technique described by Nene and Thapliyal (1993). Each fungicide was prepared at double the desired concentration by dissolving it in 50 ml of sterile distilled water. This solution was added to 50 ml of melted double-strength PDA, thoroughly mixed, and poured into Petri plates containing a pinch of streptocycline (Hindustan Antibiotic Ltd.). For the control treatment, 50 ml of sterile distilled water was mixed with 50 ml of double-strength PDA without fungicide. A 5 mm disc of each test fungus (*C. fimbriata* and *F. oxysporum*) was placed in the center of each plate with poisoned medium at different concentrations for all treatments and incubated at 25°C. Eight fungicides with a control treatments were tested and replicated thrice for each fungus, the experiment was laid out using completely randomized design (CRD) under laboratory conditions. When fungal growth in the control treatment reached the edge (90 mm), the mycelial growth in the fungicide-treated plates was measured and per cent growth inhibition was calculated by using the formula given by Vincent (1947).

$$\text{Mycelial growth inhibition (\%)} = \frac{(\text{Mycelial growth (mm)in control} - \text{Mycelial growth (mm)in treatment})}{\text{Mycelial growth (mm)in control}} \times 100$$

D. Evaluation of fungicides against C. fimbriata and F. oxysporum colonies cfu/ g soil under pot conditions

This experiment was conducted on pots containing pomegranate seedlings maintained prior to the study in the greenhouse during 2019 and 2021. During year 2019, the pots containing the healthy pomegranate seedlings inoculated with a mass multiplied inoculum of *C. fimbriata* and *F. oxysporum* @ 2g/kg soil as mentioned earlier in text (Chaudhari *et al*., 2017). After the establishment of disease symptoms or partial wilting of pomegranate seedlings soil samples were taken from each treatment for counting of pathogens colonies/g soil under laboratory conditions. Each treatment was then drenched twice at 15 days interval with fungicide solution with 2g or 2ml dosage (1litre/pot) while the control treatment were maintained with application water without any fungicide drenching on it. For consistency across treatments, colony counts were recorded exclusively from plates corresponding to the 10^{-4} dilution, as this dilution consistently provided well-isolated and countable colonies. The fungal population was expressed as colony-forming units per gram of soil (cfu/g), calculated using the following formula:

$$\text{cfu/g} = \frac{(\text{Number of colonies} \times \text{Dilution factor})}{\text{Volume of inoculum plated (ml)}}$$

The soil samples were taken again after 30 days of 2nd drenching to count the number of colonies of each fungus cfu/g soil in each treatment. The experiment were repeated in year 2021 by following the similar method and the average data on number of colonies on each treatment before and after drenching of fungicides from both year (2019 and 2021) were pooled further analyzed using statistical analysis. A significant reduction in fungal colony counts was observed in amended treatments compared to the control. The percent reduction over control was calculated using the formula:

$$\text{Percent reduction over control (\%)} = \frac{(\text{cfu in control} - \text{cfu in treatment})}{\text{cfu in control}} \times 100$$

E. Evaluation of fungicides against C. fimbriata and F. oxysporum colonies cfu/ g soil under field conditions

The efficacy of fungicides was evaluated in a wilt-infested pomegranate orchard at the University Model Farm during 2019 and 2021. During year 2019, partially wilted pomegranate trees were tagged for treatment, and the impact of fungicides on *C. fimbriata* and *F. oxysporum* (cfu/g soil) was assessed to determine their effectiveness in managing the disease under field conditions**.** The soil samples were taken from each treatment before fungicide drenching for counting of pathogens colonies in infested soil in each treatment. Fungal colonies in soil were counted through serial dilution method on specific medium as mentioned earlier. Each plant in treatment were drenched at the rate of 2g or ml/L dose where 10-15 liters of fungicide solution were drenched twice at 15 days interval on each replication according to vigour of the plant and a separate control treatment were maintained without drenching any fungicides on it. After 30 days of 2nd (second) drenching the soil samples were taken again from each treatment and the colonies of each fungal pathogen were again counted on specific medium for each. A Randomized Block Design (RBD) was used for the field experiment with three replications, while fungal colonies under *in vitro* conditions were counted using the serial dilution method following a Completely Randomized Design (CRD), with each treatment replicated three times. The experiment was repeated during the year 2021 by following the similar methodology. The average data on number of colonies on each treatment before and after drenching of

fungicides from both year (2019 and 2021) were pooled and further analyzed using statistical tools.

F. Statistical analysis

The statistical analysis of the data collected from various experiments was performed using RStudio software (Field *et al*., 2013). *In vitro* data on mycelial growth inhibition of *C. fimbriata* and *F. oxysporum* were analyzed using two-way ANOVA, with fungicide and concentration as factors. Pot and field experiment data were $\log_{10}$-transformed to normalize colony-forming unit (CFU) counts. Tukey's HSD was applied for multiple comparisons (Tukey, 1949). Two-way ANOVA was conducted to assess the effects of treatment, time, and their interaction. The Shapiro-Wilk test was used to evaluate the normality of residuals, while Levene's test assessed the homogeneity of variances (Shapiro and Wilk, 1965). All analyses were carried out using R packages rstatix (for assumption tests and ANOVA), car (for model diagnostics), agricolae (for Tukey's HSD test), and ggplot2 (for visualizations). Significant treatment effects were detected ($p < 0.05$) and visualizations were created using the ggplot2 package (Wickham, 2011).

RESULTS

A. Disease symptoms

The disease was typically observed in patches of 5–10 infested plants in an orchard, with symptoms becoming noticeable rainy season. Hot and dry summer followed by continuous rainfall, were found to be highly conducive to the development and spread of the disease. Both above- and below-ground symptoms were noticed. Above-ground symptoms included yellowing and drying of leaves, which eventually led to plant wilting. Initially, the yellowing was localized to a single branch or one side of the canopy and gradually spread to other parts of the plant with time (Fig. 1a) resulting in complete wilting or drying of full bearing pomegranate plant (Fig. 1b). The disease progression was observed more rapid in orchards with poor management practices and waterlogged basin soil that results in plant mortality in rapid succession, particularly under suboptimal management conditions. Infected plants also exhibited drying of fruit, flowers, and buds, significantly reducing productivity and overall plant health. Stems of infected plants showed distinct blue or black staining (Fig. 1c; 1d), while roots displayed notable changes, including discoloration of vascular tissues (Fig. 1e) and drying of xylem vessels (Fig. 1f). Pathogen isolation under laboratory conditions confirmed the presence of *F. oxysporum* and *C. fimbriata* being associated with these symptomatic roots and stems.

Fig. 1. Symptom of pomegranate wilt disease under field conditions; a: yellowing of leaves; b: drying of complete plant ; c;d: staining of vascular tissue of stem; e: staining of vascular tissue of roots; f: drying of root xylem vessels.

B. Pathogen Identification

Two fungal pathogens were consistently isolated from infected roots and stems, with *C. fimbriata* associated to vascular staining of roots and stem, and *F. oxysporum* linked to drying of xylem tissue of roots.

(i) *C. fimbriata*. The culture of *C. fimbriata* exhibited a grayish-white coloration with a distinct growth pattern on PDA medium and displayed a dense, single growth structure with a light brown center surrounded by grayish-white growth along the rim, characterized by pinhead-like structures and an undulating border (Fig. 2). The colony margins ranged from regular to irregular, and complete mycelial growth (90 mm) was achieved after 18 days of incubation at 25°C. The growth pattern was observed dense and mat-like, contributing to the colony's grayish-white appearance. Microscopic examination revealed that the mycelium of *C. fimbriata* consisted of septate, branched hyphae that were hyaline when young but darkened with age. Morphologically, the fungus produced oval, olive-brown endoconidia while the aleuroconidia were smaller, truncate at the base, and golden-brown (Fig. 2). The presence of black to dark-brown perithecia was observed (Fig. 4), and the ascospores were hyaline and hat-shaped.

(ii) *F. oxysporum*. The culture of *F. oxysporum* on PDA medium initially exhibited whitish mycelial growth, which later developed pale purple to reddish-pink pigments, accompanied by fluffy aerial hyphae and discrete orange sporodochia (Fig. 2). The colony margins were observed from regular to irregular, reaching a diameter of approximately 90 mm after 12

days of incubation at 25°C. Microscopic examination revealed septate, hyaline (non-pigmented), and branched mycelium. The fungus produced a large number of macroconidia and microconidia. Macroconidia, predominantly formed in sporodochia, varied in size and shape, ranging from sickle-shaped to slightly or strongly curved, with 3–5 septa (Fig. 2) while the microconidia were observed oval, elliptical, reniform, or allantoid in shape. Chlamydospores were observed as thick-walled, round to oval in shape, found singly, in pairs, or occasionally in short chains, and formed either intercalary within the hyphae or terminally at their ends.

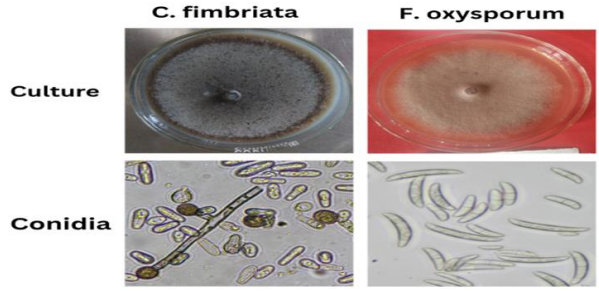

Fig. 2. Cultural and spore characteristics of *C. fimbriata* (aleuroconidia, endoconidia) and *F. oxysporum* (macroconidia, microconidia) on PDA.

C. Pathogenicity test

The pathogenicity test confirmed the virulence of both pathogens isolated from wilt-affected pomegranate plants. Under pot conditions, *C. fimbriata* showed a shorter incubation period, with leaf yellowing observed around 17 days after inoculation (DAI) and complete plant mortality by 36 DAI. In comparison, *F. oxysporum* caused symptoms more slowly, with yellowing at an average of 22 DAI and complete wilting by 38 DAI. When both pathogens were co-inoculated, disease progression accelerated, with complete wilting occurring by 35 DAI, suggesting a possible synergistic interaction that intensified disease severity. Distinct pathogenic mechanisms were observed: *C. fimbriata* caused vascular discoloration with blue-black streaks in roots, while *F. oxysporum* led to xylem drying and disruption of water transport. Re-isolation and morphological confirmation of both fungi from symptomatic tissues validated their role as the causal agents. The faster progression of symptoms in plants inoculated with both pathogens highlights the potential for enhanced disease development under co-infection conditions poses a significant challenge to disease management and necessitate a deeper understanding of their synergistic effects.

D. In vitro evaluation of fungicides against mycelial growth of C. fimbriata

The *in vitro* evaluation of fungicides against the mycelial growth of *C. fimbriata* revealed significant differences in efficacy, influenced by the mode of action and concentration. Among the systemic fungicides, Propiconazole *25%* EC demonstrated the highest average mycelial inhibition at all concentrations (100, 200, and 300 ppm), with an inhibition rate of 94.44% at 300 ppm (Fig. 3) formed a distinct Tukey group (a), indicating its superior efficacy closely followed by the combi-fungicide Carbendazim *12%* + Mancozeb *63%* WP, and Difenoconazole 25% EC, with average inhibition rates of 89.07% and 88.33% at 500ppm, respectively placed in group under Tukey group (ab), showing significant control over the pathogen (Table 1).

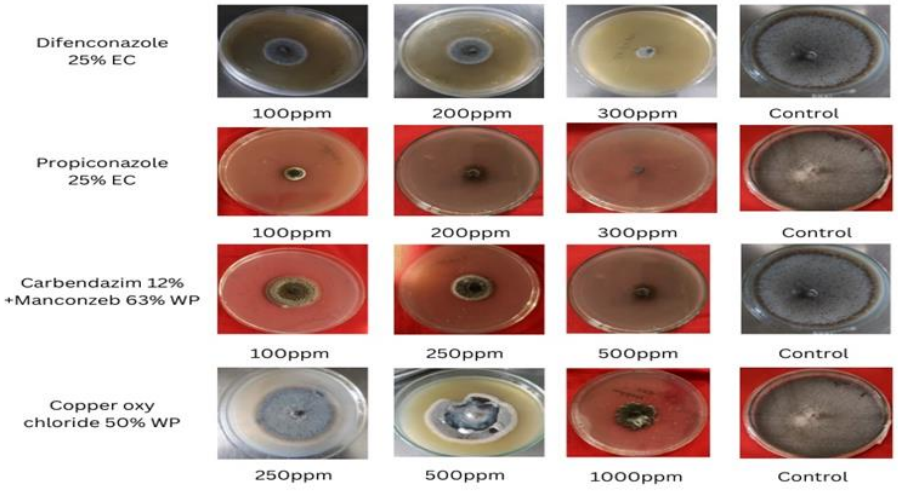

Fig. 3. In vitro inhibition of mycelial growth of *C. fimbriata* by Difenoconazole 25% EC, Propiconazole 25% EC, Carbendazim + Mancozeb WP and Copper Oxychloride 50 WP at different concentrations.

Thiophanate methyl 70% WP exhibited moderate efficacy with an average inhibition of 75.37%, also belonging to Tukey group (ab). Non-systemic fungicides, including Chlorothalonil WP, and Copper oxychloride *50%* WP, showed lower inhibition rates, ranging from 70.62% to 71.66% at 1000ppm, and formed Tukey group (c), indicating their comparatively moderate performance. Fosetyl-Al 80% WP and Metiram + Pyraclostrobin 60 WG exhibited a similar inhibition rate to the non-systemic fungicides. The untreated control showed no inhibition, forming a distinct Tukey group (d). Fig. 5 depicted mycelial

growth inhibition (%) by fungicides against *C. Fimbriata*. The ANOVA revealed significant effects of both fungicide type and concentration on the mycelial growth of *C. fimbriata* under *in vitro* conditions (Table S1, Appendix-I, supplementary data). The effect of fungicides was highly significant (**F** = 102.39, $p < 0.001$), indicating substantial differences among the treatments in suppressing fungal growth. Likewise, concentration also had a significant effect suggesting that the efficacy of fungicides varied depending on their dosage. The low residual mean square further supports the consistency of the treatment effects.

E. In vitro evaluation of fungicides against mycelial growth of *F. oxysporum*

The *in vitro* evaluation of fungicides against the mycelial growth of *F. oxysporum* revealed significant variations in efficacy depending on the fungicide type and concentration (Table 1). Among the treatments, Carbendazim 12% + Mancozeb 63% WP demonstrated the highest average inhibition (81.85%) at all three fungicide concentration tested and was classified under Tukey group (a), indicating its superior performance in controlling the pathogen (Fig. 4). Propiconazole 25% EC also exhibited strong inhibition with an average value of 72.78% and was grouped under Tukey group (ab). Other systemic fungicides, including Difenoconazole 25% EC and Thiophanate methyl 70% WP, showed moderate efficacy, with average inhibition rates of 61.36% and 57.22%, respectively, forming Tukey groups (bc and bcd). Non-systemic fungicides including Chlorothalonil WP and Copper oxychloride 50 WP, exhibited moderate to low inhibition, with averages of 48.46% and 39.38%, respectively, and were categorized under Tukey groups (cde and de). Metiram + 60 WG exhibited the lowest inhibition among the fungicides tested (36.05%, e) and was comparable to the non-systemic treatments. Figure 5 depicted nhibition of mycelial growth inhibition (%) by fungicides against *F. Oxysporum.* The untreated control treatment revealed no inhibition (f), emphasizing the necessity of fungicide application in reducing the mycelial growth of *F. oxysporum*. The ANOVA demonstrated both fungicide and concentration effects were also highly significant (Table S1, Appendix-I, supplementary data). The fungicide factor showed a strong influence on fungal inhibition (**F** = 44.45, $p < 0.001$), demonstrating notable variation in efficacy among the tested fungicides. Similarly, the effect of concentration was significant, indicating dose-dependent suppression of mycelial growth. The residual variance was slightly higher than that observed for *C. fimbriata*, suggesting relatively more variability in response.

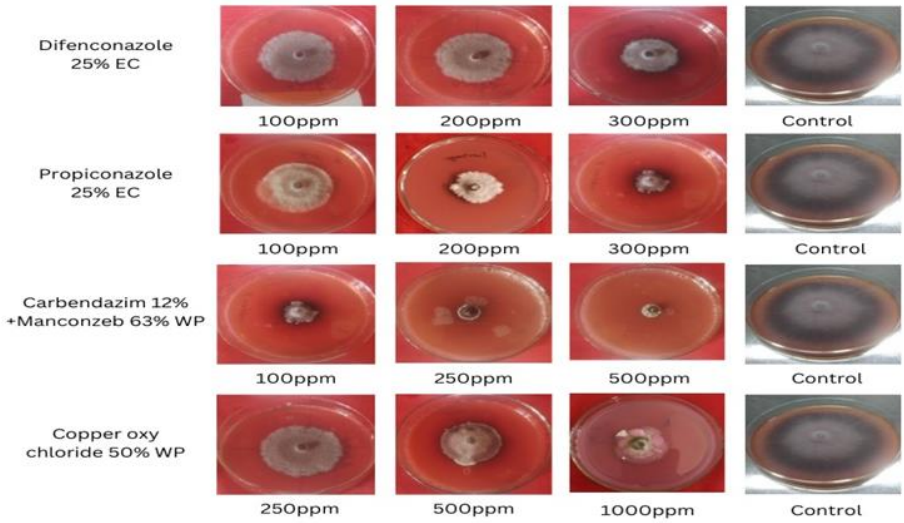

Fig. 4. *In vitro* inhibition of mycelial growth of *F. oxysporum* by Difenoconazole 25% EC, Propiconazole 25% EC, Carbendazim + Mancozeb WP and Copper Oxychloride 50 WP, at different concentrations.

Table 1: *In vitro* evaluation of fungicides against mycelial growth of *C. fimbriata* and *F. oxysporum.*

Treatments	Mycelial Inhibition *C. fimbriata* (%)					Mycelial Inhibition *F. oxysporum* (%)				
	C1	C2	C3	Mean	Tukey Group	C1	C2	C3	Mean	Tukey Group
Difenoconazole 25%EC	78.33	86.66	100	88.33	ab	53.51	59.62	70.92	61.35	bc
Propiconazole 25% EC	83.33	100	100	94.44	a	68.88	73.33	76.11	72.77	ab
Thiophanate methyl 70% WP	71.66	73.88	80.55	75.37	ab	42.40	55.55	73.70	57.22	bcd
Fosetyl-Al 80% WP	60	63.88	70.00	64.62	c	27.40	49.99	66.85	48.08	cde
Chlorothalonil WP	58.33	70.00	83.88	70.74	c	43.51	47.96	53.88	48.45	cde
Copper oxychloride 50 WP	60.55	74.44	80.00	71.66	c	28.88	39.99	49.25	39.38	de
Metiram+pyraclostrobin 60 WG	58.33	70.00	83.88	70.74	c	28.70	36.48	42.96	36.04	e
Carbendazim 12% +mancozeb 63% WP	80.55	86.66	100.00	89.074	ab	76.29	81.85	87.40	81.85	a
Control	0	0	0		d	0	0	0	0.00	f

*C1, C2, C3 for Systemic fungicides: 100, 200 and 300 ppm, Non systemic fungicides: 250, 500 and 1000ppm; Combi-fungicides; 100, 250 and 500 ppm respectively.

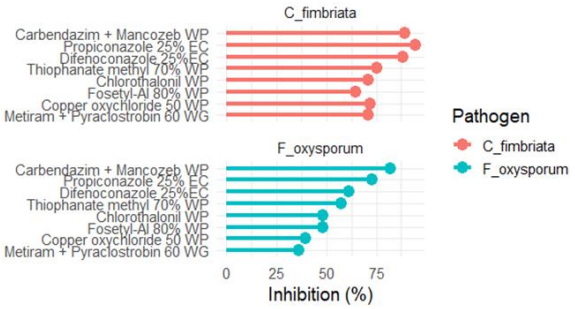

Fig. 5. Inhibition of mycelial growth (%) by fungicides against *C. fimbriata* and *F. oxysporum.*

F. Evaluation of fungicides against C. fimbriata colonies/g soil under pot conditions

Application of different fungicides significantly affected the population of *C. fimbriata* in pot soil, as reflected by changes in $\log_{10}$ cfu/g values before and after treatment revealed all fungicide treatments led to reductions (Table 2). While the control treatment showed an increase in fungal population (from 6.68 to 6.99 $\log_{10}$ cfu/g). The most effective treatment was Propiconazole 25% EC, which resulted in the highest reduction ($\Delta\log_{10}$ = –1.03) and a 95.9% decrease in population compared to the control. This was followed by Carbendazim + Mancozeb WP (–0.90 $\Delta\log_{10}$; 94.0%), Difenoconazole 25% EC (–0.84; 93.5%), and Metiram + Pyraclostrobin 60 WG (–0.75; 91.3%). Thiophanate methyl 70% WP, Fosetyl-Al 80% WP, Chlorothalonil WP, and Copper oxychloride 50 WP also showed considerable reductions. Tukey's HSD grouping indicated significant differences among treatments, with Propiconazole, Carbendazim + Mancozeb, and Difenoconazole forming distinct groups from the less effective treatments and the control. Two-way ANOVA (Table S2, Appendix-I, supplementary data) revealed highly significant effects of treatment ($F = 45.32$, $p < 2e{-}16$), time ($F = 1034.06$, $p < 2e{-}16$), and their interaction ($F = 41.38$, $p = 7.01e{-}16$) on fungal population, confirming that the reduction was both time- and treatment-dependent. Normality testing using the Shapiro–Wilk test (Table S3, Appendix-I, supplementary data) confirmed that most treatments had normally distributed residuals ($p > 0.05$), except for Fosetyl-Al and Metiram + Pyraclostrobin, which slightly deviated from normality ($p = 0.000$). Levene's test for homogeneity of variance (Table S4, Appendix-I, supplementary data) indicated that variances across treatments were homogeneous ($F = 0.6293$, $p = 0.7432$), validating the assumptions of ANOVA.

Table 2: Effect of fungicides on *C. fimbriata* population under pot condition.

Treatment	Avg cfu/g Before	Avg cfu/g After	$\log_{10}$ cfu/g Before	$\log_{10}$ cfu/g After	$\Delta\log_{10}$ cfu/g	Tukey Group	% Reduction over Control (cfu)
Difenoconazole 25% EC	4,333,333	633,333	6.64	5.80	-0.84	cde	93.5%
Propiconazole 25% EC	4,333,333	400,000	6.64	5.60	-1.03	e	95.9%
Thiophanate methyl 70% WP	4,316,667	733,333	6.64	5.87	-0.77	bcd	92.5%
Fosetyl-Al 80% WP	4,483,333	1,000,000	6.65	6.00	-0.65	bcd	89.7%
Chlorothalonil WP	4,750,000	1,150,000	6.68	6.06	-0.62	b	88.2%
Copper oxychloride 50 WP	4,316,667	1,116,667	6.64	6.05	-0.59	bc	88.6%
Metiram + Pyraclostrobin 60 WG	4,833,333	850,000	6.68	5.93	-0.75	bcd	91.3%
Carbendazim + Mancozeb WP	4,600,000	583,333	6.66	5.77	-0.90	de	94.0%
Control	4,733,333	9,750,000	6.68	6.99	+0.31	a	–

G. Evaluation of fungicides against F. oxysporum colonies/g Soil under pot conditions

Application of different fungicidal treatments had a significant effect on the soil population of *F. oxysporum*, as observed from the $\log_{10}$-transformed cfu/g values before and after treatment (Table 3). The untreated control showed an increase in the fungal population, with $\log_{10}$ cfu rising from 6.66 to 7.01, indicating active multiplication of the pathogen in the absence of any intervention. In contrast, all fungicide treatments led to a decrease in cfu levels post-application, with varying degrees of suppression among treatments. Among the tested fungicides, treatment Propiconazole 25% EC proved most effective, causing the highest reduction in population ($\Delta\log_{10}$ = –0.81) and achieving a 94.3% decrease relative to the control (Figure 6) This was closely followed by Carbendazim + Mancozeb WP (–0.74 $\Delta\log_{10}$; 93.2%) and Difenoconazole 25% EC (–0.62 $\Delta\log_{10}$; 92.1%). Copper oxychloride 50 WP was comparatively less effective, showing the lowest reduction among fungicides ($\Delta\log_{10}$ = –0.42; 85.6%). Tukey's HSD test distinguished these treatments into statistically significant groupings, where Propiconazole and Carbendazim + Mancozeb were placed in distinct groups from less effective treatments and the control. The two-way ANOVA (Table S5, Appendix-I, supplementary data) revealed that all main effects and interactions were highly significant ($p < 0.001$). Specifically, the treatment effect ($F = 70.07$), time effect ($F = 952.03$), and treatment × time interaction ($F = 50.65$) significantly influenced the $\log_{10}$-transformed cfu values of *F. oxysporum*, indicating that both the type of fungicide and the timing of application strongly determined the pathogen suppression outcome. Assumptions of ANOVA were examined through normality and homogeneity tests. The Shapiro–Wilk normality test (Table S6, Appendix-I, supplementary data) indicated that most treatments met the normality assumption ($p > 0.05$), except for Fosetyl-Al and Propiconazole, which showed deviations ($p = 0.000$), suggesting slight distributional skewness in those treatments. However, Levene's test for homogeneity of variance (Table S7, Appendix-I, supplementary data) indicated a violation of the equal

variance assumption across treatment groups ($F = 10.156$, $p = 5.82 \times 10^{-8}$), suggesting heteroscedasticity in the data. Despite this, the ANOVA results remain valid due to the robustness of the test to moderate deviations. The findings clearly demonstrate that specific fungicides—particularly Propiconazole 25% EC and Carbendazim + Mancozeb WP are highly effective in reducing *F. oxysporum* populations in soil among all treatments. The results support the potential use of these fungicides in integrated wilt management strategies targeting soil-borne Fusarium infections. The Q-Q plot comparing residuals from the two-way ANOVA models for *F. oxysporum* and *C. fimbriata* demonstrated that both datasets largely follow the normality line, indicating that residuals are approximately normally distributed (Figure 7). While minor deviations are observed at the tails, slightly more for *Fusarium* and the central distribution aligns well with the theoretical quantiles. Residuals for *Ceratocystis* show slightly better adherence to normality. Overall, the $\log_{10}$ transformation and balanced design help meet the ANOVA normality assumption, supporting the validity of the statistical analysis.

Table 3: Effect of fungicides on *F. oxysporum* population under pot condition.

Treatment	Avg cfu/g Before	Avg cfu/g After	$\log_{10}$ cfu/g Before	$\log_{10}$ cfu/g After	$\Delta\log_{10}$ cfu/g	Tukey Group	% Reduction over Control (cfu)
Difenoconazole 25% EC	3,416,667	816,667	6.53	5.91	-0.62	cde	92.1%
Propiconazole 25% EC	3,750,000	583,333	6.57	5.77	-0.81	e	94.3%
Thiophanate methyl 70% WP	3,433,333	1,033,333	6.54	6.01	-0.52	bcd	90.0%
Fosetyl-Al 80% WP	3,716,667	1,016,667	6.57	6.01	-0.56	bcd	90.1%
Chlorothalonil WP	3,900,000	1,133,333	6.59	6.05	-0.54	bc	89.0%
Copper oxychloride 50 WP	3,866,667	1,483,333	6.59	6.17	-0.42	b	85.6%
Metiram + Pyraclostrobin 60 WG	3,883,333	1,150,000	6.59	6.06	-0.53	bc	88.8%
Carbendazim + Mancozeb WP	3,816,667	700,000	6.58	5.85	-0.74	de	93.2%
Control	4,566,667	10,300,000	6.66	7.01	+0.35	a	–

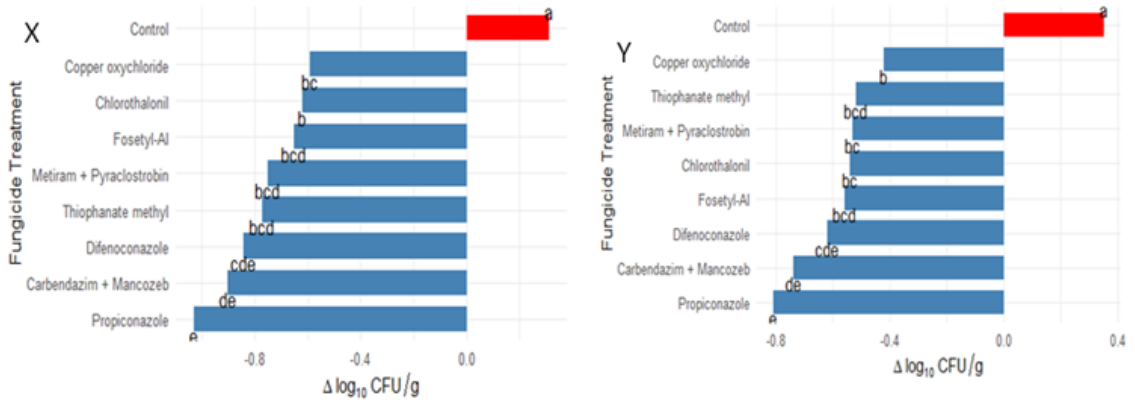

Fig. 6. Reduction in Log_{10} CFU of *C. fimbriata* (X) and *F. oxysporum* (Y) after fungicide treatment under pot conditions, Positive $\Delta\log_{10}$ CFU in the control reflects an increase in pathogen population.

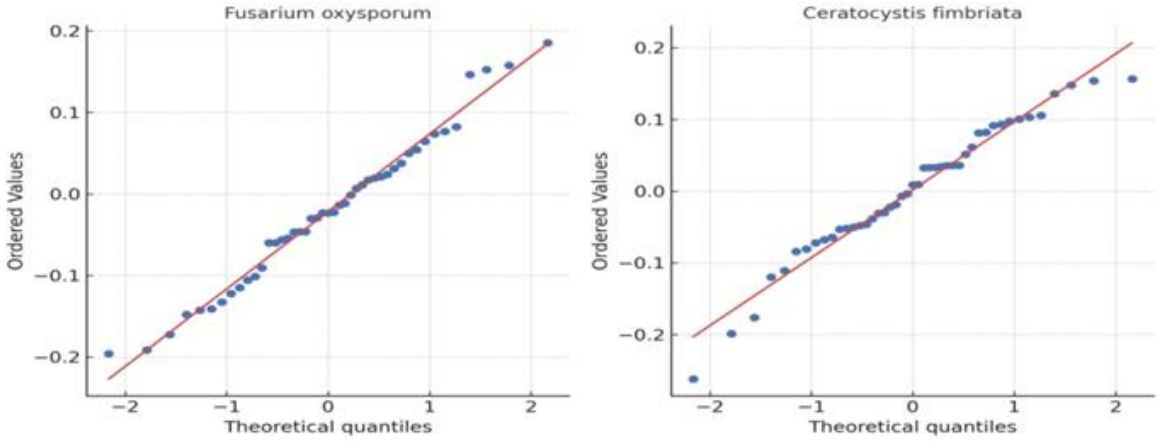

Fig. 7. Q-Q Plot of residuals from Two-Way ANOVA under pot conditions for *C. fimbriata* and *F. oxysporum* treated with fungicides.

In the pot experiment, all fungicide treatments reduced the severity of wilt symptoms in pomegranate plants. Treated plants exhibited delayed disease progression and healthier foliage compared to untreated controls treatments which demonstrated rapid disease development, severe wilting, and complete drying at later stages, leading to total plant collapse.

H. Evaluation of fungicides against C. fimbriata colonies/g soil under field condition

Application of different fungicidal treatments significantly influenced the soil population of *C. fimbriata* under field conditions, as measured by changes in log_{10}-transformed colony-forming units per gram (CFU/g) of soil (Table 4). All fungicides tested resulted in a notable reduction in pathogen population compared to the untreated control, which exhibited an increase in cfu levels post-treatment (from 6.58 to 7.03 log_{10} CFU/g, i.e., a +0.455 log unit change). Among the treatments, Propiconazole 25% EC exhibited the highest suppressive effect with a Δlog_{10} CFU of -0.778 and a corresponding 95.1% reduction over the control treatment, followed closely by Carbendazim + Mancozeb WP and Difenoconazole 25% EC, demonstrating 93.6% and 93.2% reductions, respectively (Figure 8). The two-way ANOVA (Table S8, Appendix-I, supplementary data) confirmed a highly significant effect of treatment ($F = 80.86$, $p < 2e\text{-}16$), time (before vs. after application; $F = 1936.39$, $p < 2e\text{-}16$), and their interaction ($F = 75.61$, $p < 2e\text{-}16$) on *C. fimbriata* population, indicating that not only individual fungicide efficacy but also the change over time and specific treatment-time combinations were critical in influencing fungal dynamics. Levene's test for homogeneity of variances yielded a non-significant result ($F = 0.9298$, $p = 0.5487$), validating the assumption of equal variance across groups (Table S10, Appendix-I, supplementary data) However, the Shapiro-Wilk normality test indicated that post-treatment log_{10} cfu values deviated significantly from normal distribution (Table S11, Appendix-I, supplementary data). Tukey's HSD test grouped the treatments based on their log_{10} cfu means, with the control being statistically distinct (group 'a') and highly suppressive treatments like Propiconazole and Carbendazim + Mancozeb forming separate lower groupings (group 'd' and 'cd'), reflecting their superior efficacy in suppressing *C. fimbriata* population in soil.

Table 4: Effect of fungicides on *C. fimbriata* population under field condition.

Treatment	Avg cfu/g Before	Avg cfu/g After	log_{10} cfu/g Before	log_{10} cfu/g After	Δlog_{10} cfu/g	Tukey Group	% Reduction over Control (cfu)
Difenoconazole 25% EC	4,350,000	666,667	6.52	5.82	-0.701	cd	93.2%
Propiconazole 25% EC	4,616,667	483,333	6.56	5.78	-0.778	d	95.1%
Thiophanate methyl 70% WP	4,466,667	750,000	6.53	5.92	-0.610	bc	92.4%
Fosetyl-Al 80% WP	4,500,000	1,016,667	6.54	6.01	-0.526	b	89.7%
Chlorothalonil WP	4,616,667	1,133,333	6.54	6.00	-0.542	b	88.5%
Copper oxychloride 50 WP	4,500,000	1,083,333	6.58	6.10	-0.475	b	89.0%
Metiram + Pyraclostrobin 60 WG	4,666,667	866,667	6.54	6.01	-0.529	bc	91.2%
Carbendazim + Mancozeb WP	4,516,667	633,333	6.53	5.91	-0.615	cd	93.6%
Control	4,750,000	9,850,000	6.58	7.03	+0.455	a	-

I. Evaluation of fungicides against F. oxysporum colonies/g soil under field condition conditions

Application of various fungicidal treatments significantly reduced the soil population of *F. oxysporum* compared to the untreated control (Table5). The control plots showed an increase in pathogen load, with cfu values rising from 6.58 to 7.03 log_{10} cfu/g, reflecting a +0.444 log unit change. In contrast, all fungicidal treatments led to a notable decline in fungal population. Propiconazole 25% EC exhibited the strongest suppressive effect, reducing the fungal population by -0.741 log units and achieving a 93.9% reduction relative to the control, followed closely by Difenoconazole 25% EC (-0.670 log, 93.1%) and Carbendazim + Mancozeb WP (-0.619 log, 92.0%). These results confirm the broad-spectrum efficacy of systemic and contact fungicides in managing soil-borne *F. oxysporum* under field conditions. The statistical analysis supported these observations. A two-way ANOVA (Table S9, Appendix-I, supplementary data) revealed that treatment ($F = 127.20$, $p < 2e\text{-}16$), time (before vs. after drenching; $F = 1757.80$, $p < 2e\text{-}16$), and their interaction ($F = 110.30$, $p < 2e\text{-}16$) were all highly significant, indicating that both the fungicidal interventions and the time of application had substantial and interactive effects on fungal suppression. Levene's test (Table S10, Appendix-I, supplementary data) confirmed the homogeneity the homogeneity of variances among groups ($F = 0.6335$, $p = 0.8422$), thereby validating the assumptions required for ANOVA. However, the Shapiro -Wilk test for normality of post-treatment log_{10} cfu values (Table S11, Appendix-I, supplementary data) indicated a significant deviation from normality ($W = 0.6179$, $p = 3.37e\text{-}07$), suggesting that the data distribution was skewed after treatment, possibly due to the sharp population reductions in several treatments. Tukey's HSD test further classified the treatments based on their post-drenching log_{10} cfu means. The untreated control formed a distinct group ('a'), while the most effective treatments such as Propiconazole and Difenoconazole fell into the lower groupings ('d' and 'cd'), indicating statistically significant reductions in fungal load compared to other fungicides and the control. The Q–Q plots of residuals from the ANOVA models for *C. fimbriata* and *F. oxysporum* under field conditions indicated that the assumption of normality was reasonably met (Figure 9). For *C. fimbriata*, the residuals closely followed the theoretical quantiles with only slight deviations at the extremes, suggesting that

the residuals are approximately normally distributed. In the case of *F. oxysporum*, the residuals also generally aligned with the theoretical line, though a mild deviation was observed in the upper tail, indicating a slight right-skew. However, these deviations are minimal and fall within acceptable limits for field experiment data.

Table 5: Effect of fungicides on *F. oxysporum* population under pot condition.

Treatment	Avg cfu/g Before	Avg cfu/g After	$\log_{10}$ cfu/g Before	$\log_{10}$ cfu/g After	$\Delta\log_{10}$ cfu/g	Tukey Group	% Reduction over Control (cfu)
Carbendazim 12% + Mancozeb 63% WP	3,516,667	850,000	6.55	5.93	-0.619	cd	92.0%
Chlorothalonil WP	3,500,000	966,667	6.54	5.98	-0.561	bc	90.9%
Control	3,833,333	10,633,333	6.58	7.03	**+0.444**	**a**	-
Copper oxychloride 50 WP	3,633,333	1,316,667	6.56	6.12	-0.444	b	87.6%
Difenoconazole 25%EC	3,400,000	733,333	6.53	5.86	-0.670	cd	93.1%
Fosetyl-Al 80% WP	3,433,333	1,016,667	6.54	6.01	-0.528	bc	90.4%
Metiram + Pyraclostrobin 60 WG	3,533,333	1,016,667	6.55	6.01	-0.541	bc	90.4%
Propiconazole 25% EC	3,583,333	650,000	6.55	5.81	**-0.741**	**d**	93.9%
Thiophanate methyl 70% WP	3,516,667	966,667	6.55	5.98	-0.567	bc	90.9%

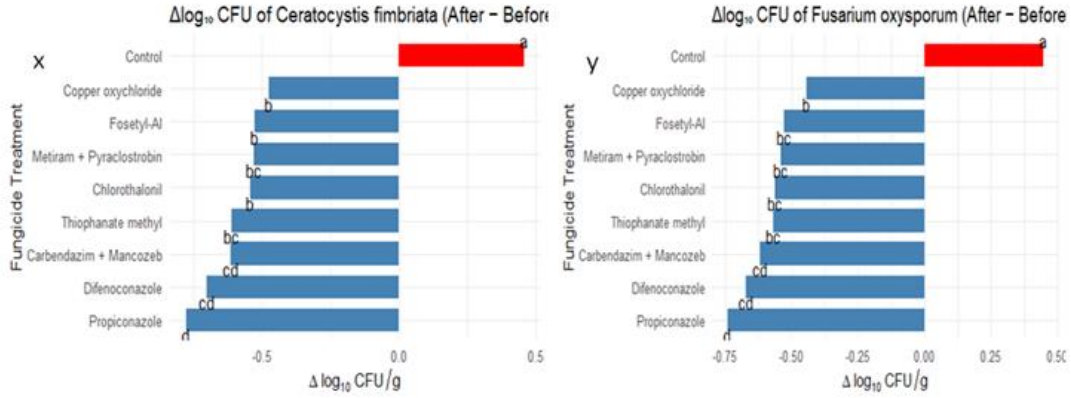

Fig. 8. Reduction in Log_{10} CFU of *C. fimbriata* (x) and *F. oxysporum* (y) after fungicide treatment under field conditions.

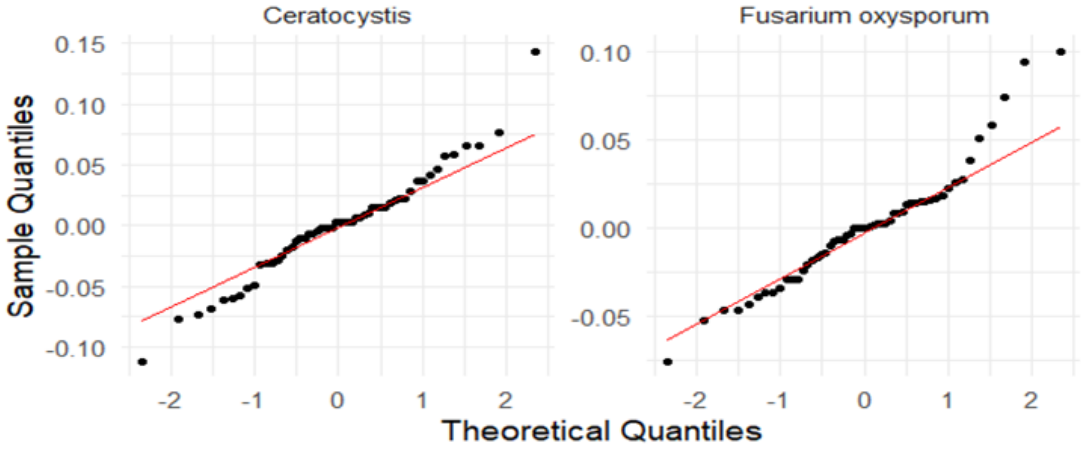

Fig. 9. Q-Q Plot of residuals from Two-Way ANOVA under field conditions for *C. fimbriata* and *F. oxysporum* treated with fungicides.

Under field conditions, each fungicide treatment led to a considerable reduction in disease severity in wilt-infected pomegranate plants. Treated plots showed improved plant vigor, delayed symptom onset, and better survival rates compared to untreated controls. Control plants, in contrast, displayed extensive wilting, progressive canopy yellowing, and ultimately dried completely during the later stages of disease development. The results demonstrate that the fungicide applications were effective in mitigating wilt under natural conditions, offering a practical approach for managing the disease in pomegranate orchard.

DISCUSSION

Pomegranate wilt, primarily caused by *C. fimbriata* and *F. oxysporum*, poses a severe threat to orchard productivity due to rapid disease progression and complete plant mortality. Initial symptoms such as

yellowing, stunting, and wilting indicate systemic vascular disruption, often resulting from fungal colonization that blocks water and nutrient flow (Huang *et al.,* 2003). *C. fimbriata* infection is distinguished by blue or black vascular streaks, while *F. oxysporum* cause browning and drying of xylem vessels, both reflecting distinct pathogenic mechanisms (Sharma *et al*., 2010; Yadeta and Thomma 2013). The post-monsoon environment and waterlogged soils likely enhance disease spread, as previously reported (Park, 1959). Morphological and cultural analyses of *C. fimbriata* matched previous descriptions, including hat-shaped ascospores and dark perithecia (Hunt, 1956; Raja *et al*., 2017), suggesting survival adaptations. The pigmentation and sporulation traits of *F. oxysporum* aligned with taxonomic descriptions by Leslie and Summerell (2006), and prior findings by Patel *et al.* (2020); Burgess *et al.* (1989) confirming its diagnostic features and adaptive capacity. Pathogenicity tests revealed that *C. fimbriata* induced faster disease onset compared to *F. oxysporum*, and co-inoculation led to even earlier wilting, indicating a synergistic interaction that amplified disease severity. These results align with earlier studies (Xu *et al*., 2011; Alam *et al*., 2016; Mohmad *et al*., 2020), reinforcing the importance of understanding dual infections for disease management.

In vitro assays demonstrated that fungicide efficacy was significantly influenced by both active ingredient and concentration. Propiconazole 25% EC was most effective against *C. fimbriata*, while Carbendazim + Mancozeb WP showed highest inhibition of *F. oxysporum.* These results were statistically supported by ANOVA ($p < 0.001$), and corroborated by previous studies (Ismail *et al*., 2018; Khan *et al.,* 2017; Khosla and Bhardwaj 2011; Sharma and Khosla 2020; Subhani *et al*., 2011; Sahar *et al.,* 2013; Maitlo *et al.,* 2014; Kubde *et al.,* 2022). Pot experiments further validated the effectiveness of these fungicides in reducing soil populations. Propiconazole 25% EC demonstrated the highest suppression, followed by Carbendazim + Mancozeb WP and Difenoconazole 25% EC while the untreated control exhibited increased pathogen loads, highlighting the fungi's aggressive nature in conducive conditions. ANOVA confirmed significant effects of treatment and time, with slight deviations from normality warranting cautious interpretation. These findings align with earlier pot trials (Ahmad *et al.,* 2021; Bhimani *et al.,* 2018; Raja *et al*., 2023). Field evaluations mirrored pot results, with systemic fungicides, particularly Propiconazole, showing strong suppression of both pathogens. The untreated control again showed pathogen proliferation, emphasizing the importance of timely interventions. Significant treatment effects and consistent group separation in Tukey's test reaffirmed these outcomes. Although some post-treatment data deviated from normality (Shapiro–Wilk), Q–Q plots and Levene's test supported the robustness of the analysis. These results validate the field applicability of systemic fungicides and support their integration into disease management strategies, in line with findings from Sonyal *et al.* (2016); Khosla (2013); Raja *et al.* (2023). In both pot and field trials, fungicide treatments significantly reduced the severity of pomegranate wilt compared to untreated controls. Treated plants exhibited delayed symptom onset, reduced vascular browning, and improved overall health and survival. In contrast, control plants showed rapid disease progression, severe wilting, and complete drying at later stages. The consistent performance of fungicides under controlled and natural conditions highlights their potential as an effective disease management strategy. These results suggest that timely application of suitable fungicides can play a crucial role in minimizing losses due to wilt, particularly in disease-prone orchards and during early stages of plant establishment. While fungicides demonstrated significant efficacy in suppressing *C. fimbriata* and *F. oxysporum* populations under both, pot and field conditions, their use for managing soil-borne pathogens poses inherent limitations. Repeated application can lead to the development of fungicide resistance, reduced soil microbial diversity, and potential environmental contamination. Additionally, systemic fungicides may have limited efficacy in reaching deeply established inoculum in the rhizosphere or in poorly drained soils. These challenges underscore the need to adopt an integrated disease management (IDM) approach that combines chemical control with cultural practices, organic amendments, resistant cultivars, and biological agents. Such a tiered strategy not only enhances disease suppression but also ensures long-term sustainability, reduces chemical dependency, and preserves soil health.

LIMITATIONS

The study was the disrupted by the COVID-19 pandemic in 2020, which led to the complete lockdown and closure of laboratories. As a result, the planned pot and field trials were delayed, with the first trial conducted in 2019 and the second-year trials postponed to 2021. This interruption affected the continuity of data collection and experimentation, potentially impacting the consistency and scope of the study. Additionally, the inability to conduct field evaluations during the critical period of disease progression in orchards limited the integration of real-time environmental factors into the findings, highlighting the challenges of conducting agricultural research during such unprecedented global disruptions.

SUPPLEMENTARY DATA
APPENDIX-I
***A. In vitro* evaluation**

Table S1: ANOVA Summary: *In vitro* evaluation of fungicides against mycelial growth of *C. fimbriata* and *F. oxysporum.*

Pathogen	Source	Df	Sum Sq	Mean Sq	F Value	Pr(>F)
C. fimbriata	Fungicide	8	18769	2346.1	102.39	2.84e-12 ***
	Concentration	2	1204	602.1	26.28	8.80e-06 ***
	Residuals	16	367	22.9		
F. oxysporum	Fungicide	8	13576	1697.0	44.45	1.73e-09 ***
	Concentration	2	1275	637.4	16.70	0.000121 ***
	Residuals	16	611	38.2		

*** = $p < 0.001$ (highly significant)

B. Pot experiments

Table S2: Two-way ANOVA showing the effect of treatment, time, and their interaction on log_{10}-transformed colony counts of *C. fimbriata.*

Source of Variation	Df	Sum of Squares	Mean Square	F Value	p-value
Treatment	8	2.011	0.251	45.32	< 2e-16 ***
Time	1	5.736	5.736	1034.06	< 2e-16 ***
Treatment × Time	8	1.836	0.230	41.38	7.01e-16 ***
Residual	36	0.200	0.006		

*** = $p < 0.001$ (highly significant)

Table S3: Normality and Homogeneity Test Results for Log_{10}-transformed CFU (After Drenching) – *C. fimbriata.*

Treatment	Shapiro-Wilk W	p-value	Normality (p > 0.05)
Carbendazim + Mancozeb	0.987	0.780	Normal
Chlorothalonil	1.000	1.000	Normal
Control	0.878	0.317	Normal
Copper oxychloride	0.980	0.726	Normal
Difenoconazole	0.980	0.726	Normal
Fosetyl-Al	0.750	0.000	Not normal
Metiram + Pyraclostrobin	0.750	0.000	Not normal
Propiconazole	0.893	0.363	Normal
Thiophanate methyl	0.923	0.463	Normal

Table S4: Levene's Test for Homogeneity of Variance – *C. fimbriata* (log_{10} cfu values).

Source	Df	F value	p-value	Homogeneity
Group	8	0.6293	0.7432	✔Variances homogeneous
Residual	18			

Table S5: Two-way ANOVA showing the effect of treatment, time, and their interaction on log_{10}-transformed colony counts of *F. oxysporum.*

Source of Variation	Df	Sum of Squares	Mean Square	F Value	p-value
Treatment	8	1.898	0.237	70.07	< 2e-16 ***
Time	1	3.224	3.224	952.03	< 2e-16 ***
Treatment × Time	8	1.372	0.172	50.65	< 2e-16 ***
Residual	36	0.122	0.003		

*** = $p < 0.001$ (highly significant)

Table S6: Shapiro-Wilk Normality Test for log_{10}-Transformed cfu Values of *F. oxysporum* (After Drenching).

Treatment	W Statistic	p-value	Normality (p > 0.05)
Carbendazim + Mancozeb	0.875	0.309	Normal
Chlorothalonil	0.997	0.899	Normal
Control	0.910	0.419	Normal
Copper oxychloride	0.862	0.272	Normal
Difenoconazole	1.000	0.960	Normal
Fosetyl-Al	0.750	0.000	Not normal
Metiram + Pyraclostrobin	0.904	0.398	Normal
Propiconazole	0.750	0.000	Not normal
Thiophanate methyl	0.977	0.707	Normal

Table S7: Levene's Test for Homogeneity of Variance *F. oxysporum* (log_{10} cfu values).

Source	Df	F value	p-value	Homogeneity
Group	8	10.156	5.82×10^{-8} (***)	Variances not homogeneous
Residual	45			

C. Field Experiment

Table S8: Two-Way ANOVA Summary for log_{10} cfu (*C. fimbriata*).

Source	Df	Sum Sq	Mean Sq	F value	Pr(>F)	Significance
Treatment	8	1.829	0.229	80.86	< 2e-16	***
Time	1	5.476	5.476	1936.39	< 2e-16	***
Treatment × Time	8	1.711	0.214	75.61	< 2e-16	***
Residuals	36	0.102	0.003			

*** = $p < 0.001$ (highly significant)

Table S9: Two-Way ANOVA Summary for log_{10} cfu (*F. oxysporum*).

Source	Df	Sum Sq	Mean Sq	F value	Pr(>F)	Significance
Treatment	8	1.723	0.2154	127.20	< 2e-16	***
Time	1	2.977	2.9772	1757.80	< 2e-16	***
Treatment × Time	8	1.495	0.1868	110.30	< 2e-16	***
Residuals	36	0.061	0.0017			

*** = $p < 0.001$ (highly significant)

Table S10: Levene's Test for Homogeneity of Variance (log_{10} cfu values).

Fungus	Df (Group)	Df (Residuals)	F value	Pr(>F)
Ceratocystis fimbriata	17	36	0.9298	0.5487
Fusarium oxysporum	17	36	0.6335	0.8422

Table S11: Shapiro-Wilk Normality Test (log_{10} CFU after drenching).

Fungus	W statistic	p-value	Normality
Ceratocystis fimbriata	0.70174	4.1e-06	Not normal
Fusarium oxysporum	0.61793	3.37e-07	Not normal

CONCLUSIONS

The present study confirms the involvement of *C. fimbriata* and *F. oxysporum* as major causal agents of wilt disease in pomegranate orchards, with *C. fimbriata* showing more aggressive pathogenic behavior under pot conditions. Co-inoculation of both pathogens resulted in accelerated disease progression, indicating a synergistic interaction that intensifies wilt severity. Morphological observations and re-isolation studies validated their pathogenicity and distinct infection strategies. *In vitro* and in vivo evaluations demonstrated that systemic fungicides, particularly Propiconazole 25% EC and Carbendazim-based formulations, significantly reduced pathogen populations and inhibited mycelial growth. However, the limitations of fungicide use including resistance development and environmental concerns that highlight the need for integrated disease management approaches. Combining chemical control with cultural practices, organic amendments, and biological agents will be critical for achieving long-term, sustainable management of pomegranate wilt disease. These findings provide a foundation for future research on integrated strategies tailored to specific agro-ecological conditions.

FUTURE SCOPE

Given the limitations of sole reliance on fungicides for managing soil-borne pathogens like *C. fimbriata* and *F. oxysporum*, future research should focus on developing integrated and eco-friendly disease management strategies. Emphasis should be placed on exploring and validating the synergistic use of biological control agents (e.g., *Trichoderma* spp., PGPR), organic amendments, resistant pomegranate varieties, and soil health-improving practices. Long-term field studies are needed to evaluate the cumulative effects of these strategies on pathogen suppression, soil microbiome resilience, and crop productivity. The development of decision-support systems and tailored management packages for different agro-climatic zones will further enhance the effective and sustainable control of pomegranate wilt disease.

Acknowledgments. We express our sincere gratitude to the Department of Plant Pathology for providing essential laboratory and technical support throughout the study. We also thank the Directorate of Research for granting access to the pomegranate orchard at the university's Model Farm, which was crucial for conducting field trials. Our appreciation is further extended to the Department of Fruit Science for their valuable assistance during the course of this research.

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How to cite this article: Mukesh, Kishore Khosla, Satish K. Sharma, Anju Sharma, Saijal Khosla and Abhishek Sharma (2025). Assessment of Fungicide Efficacy in Managing Pomegranate wilt Disease caused by *Ceratocystis fimbriata* and *Fusarium oxysporum. Biological Forum, 17*(6): 38-52.