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Research Article

In vitro and *in vivo* Evaluation of Phytoextracts for the Management of Citrus Scab Caused by *Elsinoe fawcettii*

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ABSTRACT

Citrus scab is a major fungal disease limiting citrus production, necessitating environmentally safe alternatives to synthetic fungicides. This study evaluated the efficacy of selected phytoextracts under *in vitro* and *in vivo* conditions. *In vitro* assays demonstrated that all extracts significantly inhibited fungal growth, with neem showing the highest and most consistent inhibition across concentrations and time intervals. At S/2, neem achieved up to 62.04% inhibition at 5 days, increasing to 70.70% at 9 days, followed by eucalyptus and garlic, while cinnamon, turmeric, ginger, and thyme showed moderate to weak activity. Tea tree, oregano, and aloe vera were least effective. *In vivo* evaluation revealed that neem substantially reduced disease severity across all tested citrus cultivars, lowering infection levels to approximately 5-10%, thereby classifying most responses as resistant. Garlic and eucalyptus provided moderate disease suppression, resulting in resistant to tolerant responses depending on cultivar and concentration. Multivariate analyses (cluster analysis and PCA) confirmed strong genotype-dependent responses and consistently identified neem treatments as the most stable and effective across cultivars, while eucalyptus showed greater variability and garlic intermediate performance. FTIR characterization of neem, eucalyptus, and garlic extracts confirmed the presence of phenolics, flavonoids, terpenoids, and sulfur-containing compounds, supporting their antifungal potential. In conclusion, neem extract exhibited superior and broad-spectrum antifungal efficacy against citrus scab, with strong potential for development as a sustainable plant-based disease management strategy.

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Introduction

Citrus spp. (family Rutaceae, subfamily Aurantioideae) comprise several economically important fruit crops, including sweet orange (*Citrus sinensis*), lemon (*C. limon*), and mandarin (*C. reticulata*). The genus exhibits complex taxonomy, with up to 36 species recognized under different classification systems. Citrus fruits are widely cultivated across tropical, subtropical, and Mediterranean regions, with major production zones in Asia, the Americas, and the Mediterranean basin (Hazarika, 2024). They are rich sources of vitamin C, dietary fiber, flavonoids, carotenoids, and essential minerals, making them highly valuable for human nutrition and in the prevention of chronic diseases (Johnson et al., 2025). In Pakistan, citrus exports contribute approximately 2.48% to the agricultural GDP,

with export performance primarily driven by export volume, export prices, and exchange rate fluctuations (Siddique et al., 2024).

Globally, citrus cultivation covers approximately 9.5 million hectares, with annual production exceeding 147 million metric tons, making it one of the most significant fruit crops in world horticulture. Major producers include China, Brazil, the United States, South Africa, Peru, Turkey, and Morocco. Despite high production levels, postharvest losses remain substantial, particularly in countries such as Pakistan, Indonesia, and Peru, where mechanical injuries and fungal rots during handling and storage significantly reduce marketable yield (Dwiastuti et al., 2021; Pérez Romero et al., 2021; Gonzatto and Scherer Santos, 2023).

Optimal citrus growth and fruit quality depend on the adequate supply of macro- and micronutrients, including N, P, K, Ca, Mg, Fe, Zn, Cu, and Mn. These nutrients regulate canopy development, photosynthetic efficiency, peel pigmentation, juice composition, and resistance to pathogens. Deficiencies in essential nutrients compromise both physical and biochemical defense mechanisms, rendering citrus trees more susceptible to foliar and fruit pathogens. Nutrient stress therefore plays a critical role in disease susceptibility (Zekri and Obreza, 1969). Conversely, pathogen infection can disrupt nutrient uptake and translocation, disturb ionic balance, and further exacerbate disease severity in nutritionally deficient plants compared with well-nourished ones (Shabbir et al., 2022; Tripathi et al., 2022).

Elsinoë fawcettii (anamorph *Sphaceloma fawcettii*), the causal agent of citrus scab, is a major constraint in humid subtropical citrus-growing regions. The disease is characterized by corky, wart-like lesions on young leaves, twigs, and fruits, resulting in significant reductions in external fruit quality and marketability. Wet spring flushes with abundant young susceptible tissues strongly favor disease development (Whiteside, 1975). The pathogenicity of *E. fawcettii* is associated with the production of elsinochromes, photoactivated perylene quinones that generate reactive oxygen species, induce lipid peroxidation, and contribute to necrotic lesion formation. In susceptible hosts such as satsuma mandarin, symptom development occurs rapidly following infection (Shin et al., 2021; Van Heerden et al., 2024).

Effective disease management is essential to minimize yield and quality losses caused by plant pathogens. Plant growth regulators, botanical extracts, nutritional amendments, and synthetic fungicides all play important roles in citrus disease management. Citrus scab markedly reduces fruit market value by producing conspicuous surface blemishes (Agrios, 2005). Traditionally, the disease has been managed using synthetic fungicides. Sterol biosynthesis inhibitors and protective fungicides such as benomyl, ferbam, thiram, captafol, and copper-based compounds have shown effectiveness in controlling citrus scab, particularly when applied during susceptible growth stages. However, despite their short-term efficacy, these chemicals present several challenges, including the development of fungicide resistance. In Florida, benomyl-resistant strains of *E. fawcettii* are widespread, and similar resistance risks exist in other citrus-producing regions globally (Dewdney, 2025).

In response to these limitations, the present study was designed to explore eco-friendly integrated disease management strategies (Islam et al., 2024). Phytoextracts represent a sustainable alternative for plant disease control and consist of bioactive compounds derived from plants with demonstrated antifungal activity (Upadhayay et al., 2024). Plant extracts are increasingly recognized as natural fungicides due to their effectiveness against fungal pathogens and their relatively low environmental and human toxicity (Al-Samarrai et al., 2012; Nxumalo et al., 2021). Growing interest in sustainable agriculture has intensified research on botanical pesticides as viable alternatives to synthetic chemicals, with plant extracts showing strong potential for managing diverse plant pathogens (Kushaha et al., 2024). Phytoextracts act through multiple mechanisms of action, thereby reducing the likelihood of resistance development compared with single-site synthetic fungicides (Islam et al., 2024). This multi-target activity makes them a promising strategy for managing pathogen resistance and supporting long-term agricultural sustainability (Nxumalo et al., 2021; Deresa and Diriba, 2023).

The present study aimed to evaluate the antifungal efficacy of selected phytoextracts for citrus scab management in ten citrus varieties. Specifically, it assessed their inhibitory effects on *E. fawcettii* under *in vitro* conditions and their effectiveness in reducing disease severity under greenhouse conditions.

Materials and Methods

Sample collection

Citrus leaves, fruits, and twigs exhibiting scab lesions, along with symptom-free healthy samples, were collected from orchards in Bhalwal, Kot Momin, Sargodha, Faisalabad, Sahiwal, and Toba Tek Singh. The samples were washed thoroughly with tap water to remove surface debris and subsequently stored at 4 °C until further processing.

Fungal isolation and culture

For isolation of the citrus scab pathogen, 2-mm tissue segments were excised from the margins of lesions on diseased samples. The segments were surface-sterilized in 1% sodium hypochlorite for 1 min, rinsed three times with sterile distilled water, and air-dried under aseptic conditions. Potato Dextrose Agar (PDA) was prepared according to the formulation given in Table 1, sterilized by autoclaving at 121°C and 15 psi for 15 min, and poured into sterile Petri plates. The sterilized tissue segments were aseptically transferred onto PDA plates. Cultures were incubated at 25 ± 2°C under a 12-h photoperiod for 5 days. Developing colonies of *Elsinoë fawcettii* were sub-cultured to obtain pure cultures and maintained on PDA slants at 4°C. Pathogenicity was confirmed by fulfilling Koch's postulates using detached leaf assays.

Table 1. Composition of potato dextrose agar (PDA) medium.

Ingredient	Quantity
Dextrose	20 g
Agar	20 g
Potato Extract (200 g boiled in 1000 mL)	1000 mL

Preparation of phytoextracts

Ten plant species with previously reported antifungal activity, *Aloe vera*, Cinnamon (*Cinnamomum* sp.), Eucalyptus (*Eucalyptus* sp.), Garlic (*Allium sativum*), Ginger (*Zingiber officinale*), Neem (*Azadirachta indica*), Oregano (*Origanum vulgare*), Tea Tree (*Melaleuca alternifolia*), Thyme (*Thymus vulgaris*), and Turmeric (*Curcuma longa*), were used. Plant materials were shade-dried, ground into a fine powder, and extracted by maceration in 80% ethanol (1:10, w/v) for 72 h with intermittent shaking. The extracts were filtered through muslin cloth followed by Whatman No. 1 filter paper and concentrated under reduced pressure using a rotary evaporator at 40°C. Crude extracts were dissolved in 2% DMSO to prepare a stock solution (S) of 100 mg mL^{-1} (10% w/v). Serial dilutions of S/2, S/4, and S/8 corresponding to 50, 25, and 12.5 mg mL^{-1}, respectively, were prepared for further use.

In vitro evaluation (poisoned food technique)

Extracts at different concentrations were incorporated into PDA prior to solidification (45-50°C). A 5-mm mycelial disc of *E. fawcettii* was placed at the center of each Petri plate and incubated at 25 ± 2°C. Colony diameters were recorded on days 5, 7, and 9. Percentage growth inhibition was calculated relative to the untreated control using the given below formula. Each treatment was replicated three times.

$$\text{Percent Growth Inhibition} = \frac{(\text{Control growth} \times \text{Treatment growth})}{\text{Control growth}} \times 100$$

In vivo evaluation (greenhouse trials)

The three most effective extracts selected from *in vitro* screening were evaluated under greenhouse conditions on ten susceptible citrus cultivars: *Citrus sinensis* (sweet orange), *C. paradisi* (grapefruit), *C. reticulata* cv. Feutral's Early, *C. medica* (citron), *C. limonia* cv. Mayer lemon, *C. sinensis* cv. Jaffa, *C. sinensis* cv. Ruby Red, *C. sinensis* cv. Valencia Late, *C. paradisi* cv. Foster, and *C. paradisi* cv. Shamber. Plants were grown under recommended agronomic practices in a Randomized Complete Block Design (RCBD) with three replicates. Each replicate consisted of five uniform three-year-old potted plants per cultivar in 12-inch diameter pots.

Artificial inoculation was performed by spraying a conidial suspension (1×10^6 conidia mL^{-1}) using a hand atomizer for three consecutive weekly intervals. Control plants were sprayed with sterile distilled water. After inoculation, plants were covered with transparent polyethylene sheets to maintain 85 ± 5% relative humidity at 25°C until symptom development. Phytoextract sprays at the respective test concentrations were applied 24 h after inoculation.

Disease assessment

Disease severity was recorded using a modified rating scale described in Table 2 (Imran et al., 2015).

FTIR analysis

Dried crude extracts of *A. indica*, *Eucalyptus* sp., and *A. sativum* were subjected to Fourier Transform Infrared (FTIR) spectroscopic analysis. Crude extracts were concentrated using a rotary evaporator (Heidolph Laborota 4000) at 40-45°C and reduced to dryness. The

semi-solid residues were further dried in a hot-air oven at 45°C to remove residual moisture, with minimized exposure time for garlic to preserve thermolabile organosulfur compounds.

For Attenuated Total Reflectance (ATR) measurements, approximately 1-2 mg of dried solid extract or 1-2 µL of oil was directly placed on a diamond ATR crystal and pressed using the pressure arm. The crystal was thoroughly cleaned with isopropanol between successive samples. For confirmatory spectral fingerprinting, potassium bromide (KBr) pellet technique was also employed, in which ~1-2 mg of dried sample was homogenized with ~100-150 mg of spectroscopic-grade, pre-dried KBr and compressed at ~8-10 tons to form 13 mm pellets. Pellets were analyzed immediately to minimize moisture absorption.

FTIR spectra were recorded using a PerkinElmer Spectrum Two FTIR spectrometer (PerkinElmer, USA) in transmittance mode over the range of 4000-400 cm^{-1} (or 4000-500 cm^{-1}), at a resolution of 4 cm^{-1} with 32 co-added scans per spectrum. A fresh background spectrum was collected before each measurement, and atmospheric H_2O and CO_2 corrections were applied using the instrument software (Spectrum). The resulting spectra were baseline-corrected and smoothed using the Savitzky-Golay algorithm. Peak positions were assigned by comparison with published FTIR literature and spectral libraries to identify characteristic functional groups associated with phenolics, terpenoids, and organosulfur compounds.

Table 2. Disease rating scale for the assessment of citrus scab disease (Imran et al., 2015).

Grade	Disease Intensity	Response
0	00.00	Immune (I)
1	0.01-5.00	Highly Resistant (HR)
3	5.01-10.00	Resistant (R)
5	10.01-15.00	Tolerant (T)
7	15.01-25.00	Susceptible (S)
9	>25.00	Highly Susceptible (HS)

Statistical analysis

Experiments under *in vitro* conditions were conducted using a Completely Randomized Design (CRD) with three replicates per treatment. Greenhouse experiments were arranged in a Randomized Complete Block Design (RCBD) with three replications. Data were analyzed using analysis of variance (ANOVA), and treatment means were compared using Tukey's HSD test at a 5% significance level ($p < 0.05$). Multivariate analyses, including hierarchical clustering (Ward.D2 method) and Principal Component Analysis (PCA), were performed on standardized Disease severity data to assess similarity patterns among varieties and treatment responses using R software.

Results

***In vitro* evaluation of phytoextracts against citrus scab**

After 5 days, neem extract exhibited the highest inhibition at the S/2 concentration (62.04%), with all other extracts showing significantly lower inhibition compared to neem ($P < 0.05$). Significant differences were observed among treatments, where eucalyptus (55.47%) and garlic (50.63%) followed neem, while cinnamon (42.39%) and turmeric (34.06%) showed moderate inhibition. Lower inhibition was recorded for ginger (30.06%) and thyme (24.14%), whereas tea tree (17.28%), oregano (13.86%), and aloe vera (6.27%) were the least effective.

At the S/4 concentration, neem again showed the highest inhibition (60.68%), followed by eucalyptus (52.73%) and garlic (46.07%). Cinnamon (41.49%) and turmeric (33.85%) exhibited moderate inhibition, while ginger (29.43%) and thyme (21.74%) were less effective. The lowest inhibition was observed with tea tree (16.42%), oregano (9.50%), and aloe vera (5.63%).

At the S/8 concentration, neem extract maintained the highest inhibition after 5 days (53.94%), followed by eucalyptus (49.59%) and garlic (41.98%). Cinnamon (29.47%) and turmeric (26.60%) showed moderate effects, while ginger (19.50%) and thyme (16.24%) were weakly effective. Tea tree (4.09%), oregano (3.61%), and aloe vera (3.40%) exhibited negligible inhibition (Figure 1).

After 7 days, neem again recorded the highest inhibition at S/2 (67.02%), followed by eucalyptus (61.33%) and garlic (57.10%). Moderate inhibition was observed for cinnamon (49.95%) and turmeric (42.70%), while ginger (39.24%) and thyme (34.11%) showed lower efficacy. The least inhibition was recorded for tea tree (28.12%), oregano (25.17%), and aloe vera (18.57%). A similar trend was observed at S/4 and S/8 (Figure 2).

After 9 days, neem maintained the highest inhibition at S/2 (70.70%), followed by eucalyptus (65.65%) and garlic (61.91%). Cinnamon (55.54%) and turmeric (49.11%) showed moderate effects, whereas ginger

(46.03%) and thyme (41.48%) were comparatively less effective. Tea tree (36.17%), oregano (33.55%), and aloe vera (20.55%) recorded the lowest inhibition. Neem remained significantly superior at S/4 and S/8 concentrations as well (Figure 3).

Overall, neem consistently exhibited the strongest inhibitory effect across all concentrations and time intervals, followed by eucalyptus and garlic. Cinnamon and turmeric showed moderate activity, ginger and thyme displayed weak to moderate effects, while aloe vera, oregano, and tea tree were the least effective. Inhibition generally increased from day 5 to day 9, particularly in neem, eucalyptus, and garlic treatments. Mean values are summarized in Table 3.

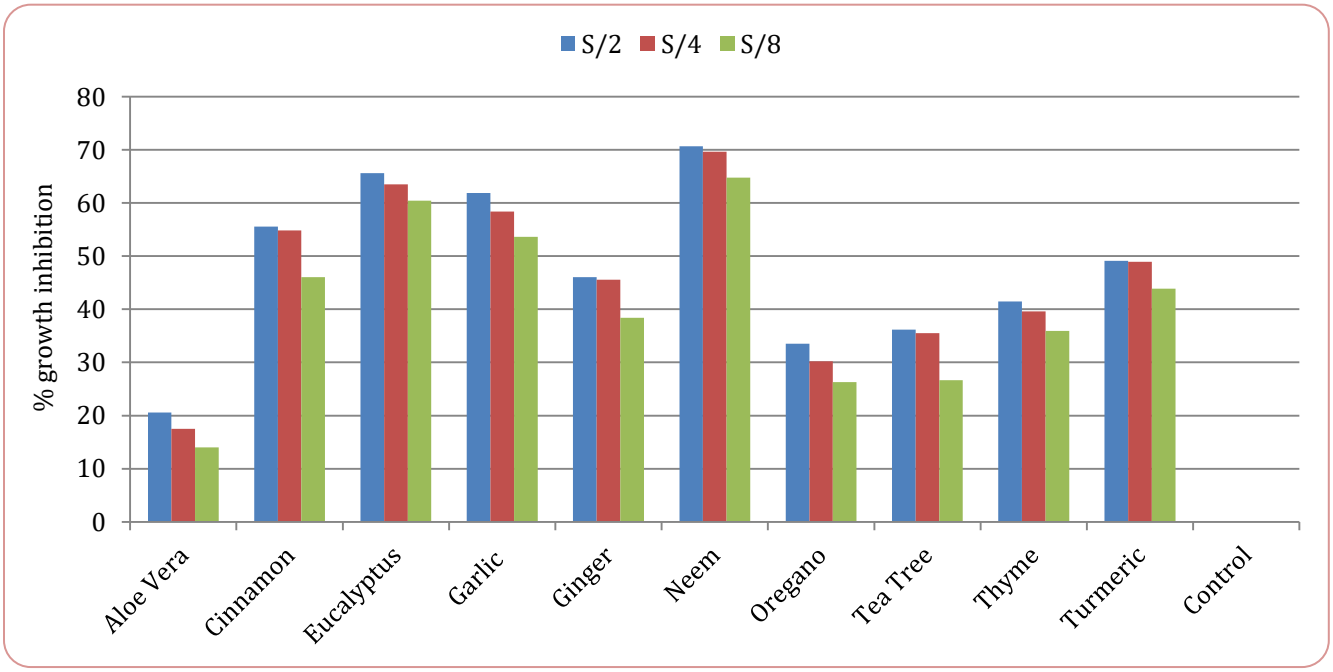

Figure 1. *In vitro* mycelial inhibition by phytoextracts after 5 days at different concentrations.

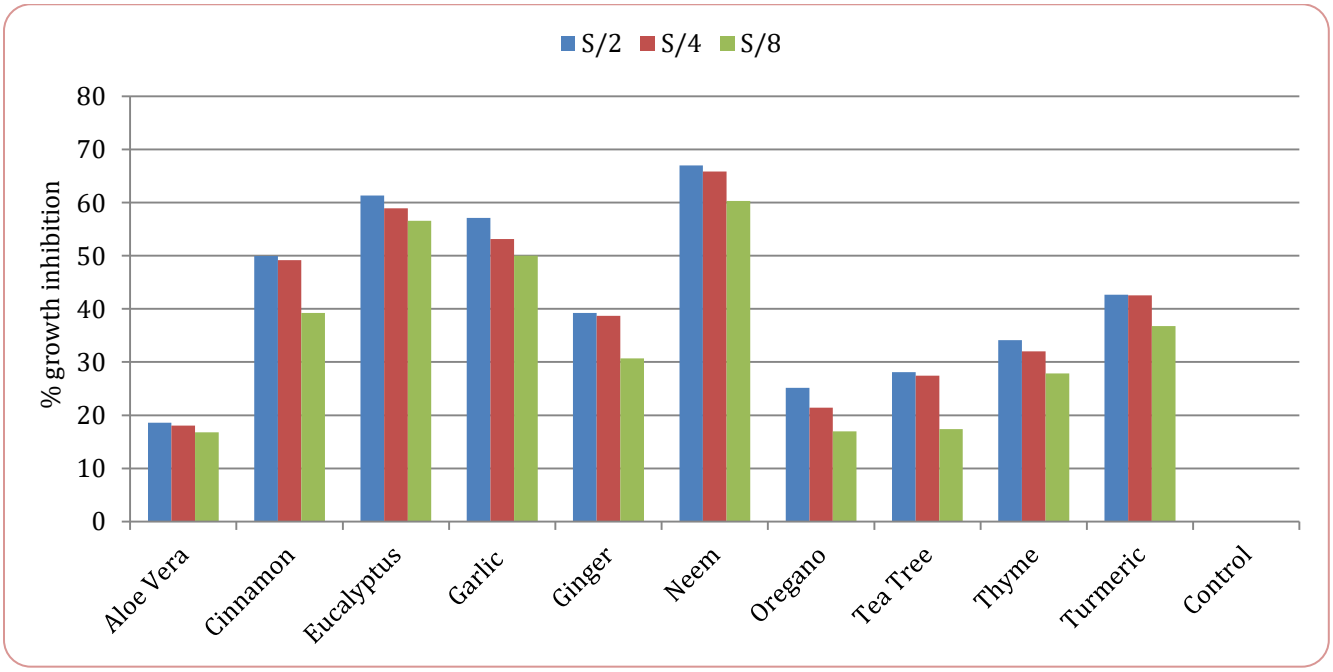

Figure 2. *In vitro* mycelial inhibition by phytoextracts after 7 days at different concentrations.

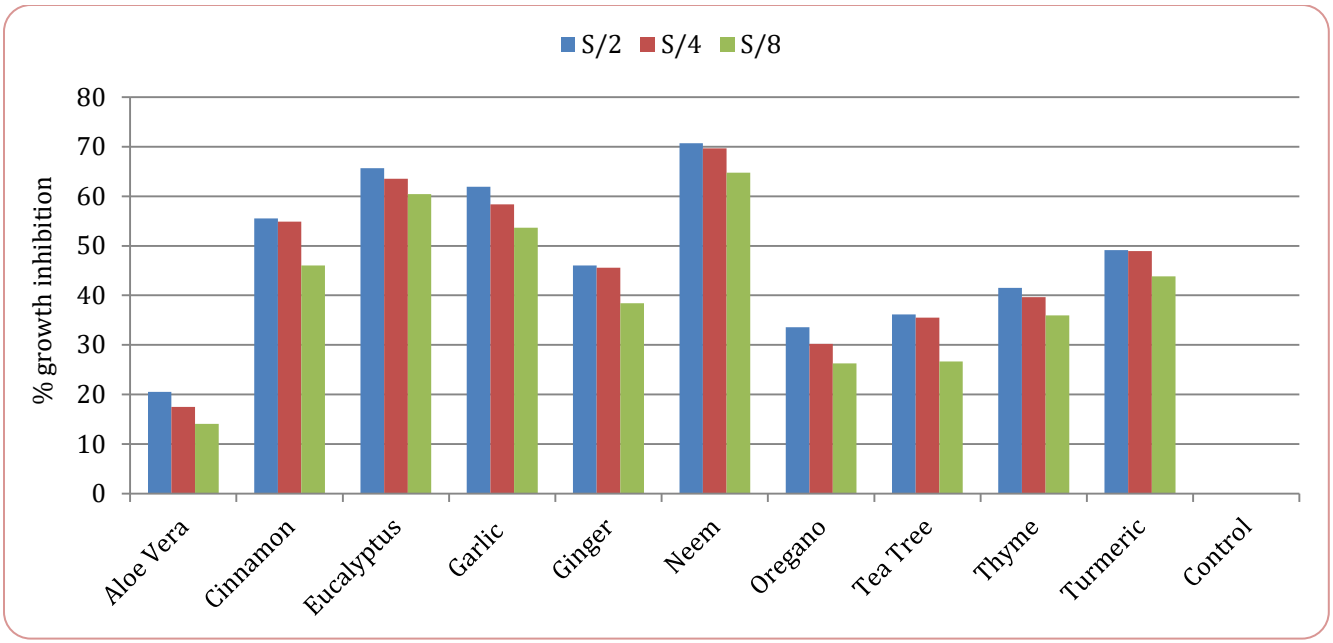

Figure 3. *In vitro* mycelial inhibition by phytoextracts after 9 days at different concentrations.

Table 3: Mean percent inhibition of *E. fawcettii* mycelial growth by plant extracts under *in vitro* conditions.

	Averaged Percent Growth Inhibition (%)								
Plant	5 Days			7 Days			9 Days		
Extracts	S/2	S/4	S/8	S/2	S/4	S/8	S/2	S/4	S/8
Aloe Vera	6.270^{u}	5.630^{u}	3.400^{w}	18.570^{t}	18.046^{t}	16.790^{u}	20.550^{s}	17.478^{t}	14.027^{u}
Cinnamon	42.396^{i}	41.496^{j}	29.474^{l}	49.952^{h}	49.188^{i}	39.256^{k}	55.548^{g}	54.866^{g}	46.060^{j}
Eucalyptus	55.470^{c}	52.730^{e}	49.594^{g}	61.330^{c}	58.932^{e}	56.566^{f}	65.650^{b}	63.518^{c}	60.446^{e}
Garlic	50.638^{f}	46.072^{h}	41.984^{ij}	57.108^{f}	53.166^{g}	50.028^{h}	61.910^{d}	58.402^{f}	53.631^{h}
Ginger	30.066^{l}	29.434^{l}	19.504^{p}	39.244^{k}	38.710^{k}	30.670^{o}	46.038^{j}	45.558^{j}	38.424^{n}
Neem	62.040^{a}	60.678^{b}	53.948^{d}	67.022^{a}	65.866^{b}	60.332^{d}	70.702^{a}	69.678^{a}	64.782^{b}
Oregano	13.862^{s}	9.504^{t}	3.616^{vw}	25.176^{r}	21.408^{s}	16.968^{u}	33.550^{p}	30.188^{q}	26.272^{r}
Tea Tree	17.282^{q}	16.420^{r}	4.096^{v}	28.126^{p}	27.418^{q}	17.392^{u}	36.172^{o}	35.524^{o}	26.646^{r}
Thyme	24.144^{n}	21.740^{o}	16.246^{r}	34.116^{m}	32.036^{n}	27.860^{pq}	41.488^{l}	39.626^{m}	35.938^{o}
Turmeric	34.056^{k}	33.850^{k}	26.604^{m}	42.698^{j}	42.552^{j}	36.780^{l}	49.110^{i}	48.970^{i}	43.860^{k}
Control	0^{x}	0^{x}	0^{x}	0^{v}	0^{v}	0^{v}	0^{v}	0^{v}	0^{v}
HSD Value	0.6777			0.619			1.0644		

Means within columns followed by the same superscript letter are not significantly different at α = 0.05.

***In vivo* evaluation against citrus scab**

In *C. sinensis*, the control exhibited 18.60% disease severity, categorizing it as susceptible. Neem treatments reduced severity to 5.40%, 5.61%, and 6.48% at S/2, S/4, and S/8, respectively, classifying the cultivar as resistant. Garlic resulted in slightly higher severity (7.08-8.61%), while eucalyptus recorded 6.39-7.37%, both indicating resistance across all concentrations.

In *C. paradisi*, the control showed 20.23% severity (susceptible). Neem reduced severity to 5.89-7.06%, garlic to 7.71-9.38%, and eucalyptus to 6.94-7.98%, with all treatments eliciting resistant responses. In *C. reticulata* cv. Feutral's Early, the control recorded 21.91% severity (susceptible). Neem significantly reduced severity to 6.38-7.67% (resistant). Garlic resulted in 8.36-10.16%, with S/8 shifting to a tolerant response, whereas eucalyptus showed resistance at S/2 and S/4 and tolerance at S/8.

In *C. medica*, control severity reached 23.73% (susceptible). Neem reduced severity to 6.91-8.30% (resistant). Garlic recorded 9.04-11.00%, showing

tolerance at S/8. Eucalyptus exhibited resistance at S/2 and S/4 and tolerance at S/8. In *C. limonia*, control severity was 24.55% (susceptible). Neem (7.15-8.59%) maintained resistance across all concentrations. Garlic (9.35-11.37%) showed resistance at S/2 and tolerance at S/4 and S/8, while eucalyptus showed resistance at S/2 and S/4 and tolerance at S/8.

In *C. sinensis* cv. Jaffa, control plants showed 27.48% severity (highly susceptible). Neem reduced severity to 7.99-9.60% (resistant). Garlic resulted in 10.46-12.72% (tolerant), whereas eucalyptus ranged from 9.42-10.82%, showing resistance at higher concentrations and tolerance at S/8. In *C. sinensis* cv. Ruby Red, control severity was 28.59% (highly susceptible). Neem reduced it to 8.31-9.99% (resistant). Garlic showed 10.87-13.22% (tolerant), while eucalyptus shifted from resistance at S/2 and S/4 to tolerance at S/8.

In *C. paradisi* cv. Foster, the control recorded 30.02% severity (highly susceptible). Neem reduced severity to 8.73-10.49%, showing resistance at S/2 and S/4 and tolerance at S/8. Garlic and eucalyptus both exhibited tolerant responses across all concentrations. In *C. paradisi* cv. Shamber, the control showed 31.95% severity (highly susceptible). Neem induced resistance at S/2 and S/4 and tolerance at S/8, while garlic and eucalyptus consistently remained in the tolerant category.

In *C. sinensis* cv. Valencia Late, the highest control severity (34.0%) was recorded, indicating a highly susceptible response. Neem showed resistance at S/2 and S/4 and tolerance at S/8, whereas garlic and eucalyptus remained in the tolerant category (Table 4; Figures 4, 5, 6).

Table 4. Mean disease severity across citrus varieties due to phytoextracts in comparison to control.

Variety	Neem			Garlic			Eucalyptus			Control
	S/2	S/4	S/8	S/2	S/4	S/8	S/2	S/4	S/8	
Sweet Orange	5.40^{H}	5.61^{H}	6.48^{F}	7.08^{BC}	7.72^{w}	8.61^{qrs}	6.39^{F}	6.78^{DE}	7.37^{yzA}	18.60^{J}
Grapefruit	5.89^{G}	6.11^{G}	7.06^{BC}	7.71^{w}	8.42^{rst}	9.38^{kl}	6.94^{BCD}	7.36^{yzA}	7.98^{v}	20.23^{I}
Feutral's early	6.38^{F}	6.63^{EF}	7.67^{wx}	8.36^{stu}	9.12^{mno}	10.16^{de}	7.53^{wxy}	7.98^{v}	8.65^{qr}	21.91^{H}
Citron	6.91^{CD}	7.18^{zAB}	8.30^{tu}	9.04^{no}	9.87^{fgh}	11^{Zab}	8.15^{uv}	8.64^{qr}	9.37^{klm}	23.73^{G}
Mayer lemon	7.15^{ABC}	7.43^{xyz}	8.59^{qrs}	9.35^{klm}	10.20^{de}	11.37^{WXY}	8.43^{rst}	8.94^{op}	9.69^{ghi}	24.55^{F}
Jaffa	7.99^{v}	8.31^{tu}	9.60^{ijk}	10.46^{c}	11.40^{WX}	12.72^{OP}	9.42^{jkl}	9.99^{ef}	10.82^{b}	27.48^{E}
Ruby red	8.31^{tu}	8.64^{qr}	9.99^{ef}	10.87^{b}	11.84^{TU}	13.22^{N}	9.78^{fghi}	10.36^{cd}	11.22^{XYZ}	28.59^{D}
Foster	8.73^{pq}	9.07^{no}	10.49^{c}	11.41^{WX}	12.41^{Q}	13.86^{M}	10.27^{cd}	10.88^{b}	11.78^{TUV}	30.02^{C}
Shamber	9.29^{lmn}	9.64^{hij}	11.14^{YZa}	12.12^{RS}	13.18^{N}	14.71^{L}	10.91^{ab}	11.55^{VW}	12.51^{PQ}	31.95^{B}
Valencia late	9.90^{fg}	10.28^{cd}	11.90ST	12.91^{O}	14.05^{M}	15.67^{K}	11.61^{UVW}	12.30^{QR}	13.32^{N}	34.00^{A}
HSD Value					0.2566					

Means within columns followed by the same superscript letter are not significantly different at α = 0.05.

Multivariate analysis

To further elucidate the responses of ten citrus varieties to different phytoextract treatments (across all concentrations), multivariate analyses, including hierarchical clustering and Principal Component Analysis (PCA), were performed. These analyses provide a comprehensive understanding of the relationships among varieties and treatments, complementing the univariate PDI results.

The hierarchical clustering dendrogram (Figure 7), based on Ward's D2 method using scaled data, identified two main clusters of citrus varieties according to their responses to the treatments. The first cluster comprised Jaffa, Ruby Red, Valencia Late, Foster, and Shamber, which were previously categorized as susceptible or highly susceptible during the initial pathogenicity assessment. This clustering indicates a relatively similar level of disease susceptibility and comparable responses to the applied phytoextracts among these varieties.

The second cluster included Sweet Orange, Grapefruit, Feutral's Early, Citron, and Mayer Lemon, which exhibited moderate baseline disease levels and showed relatively consistent reductions in disease severity across the different treatments. Overall, this grouping aligns well with the trends observed in both *in vitro* and *in vivo* experiments, reinforcing that disease susceptibility and treatment efficacy are strongly cultivar-dependent.

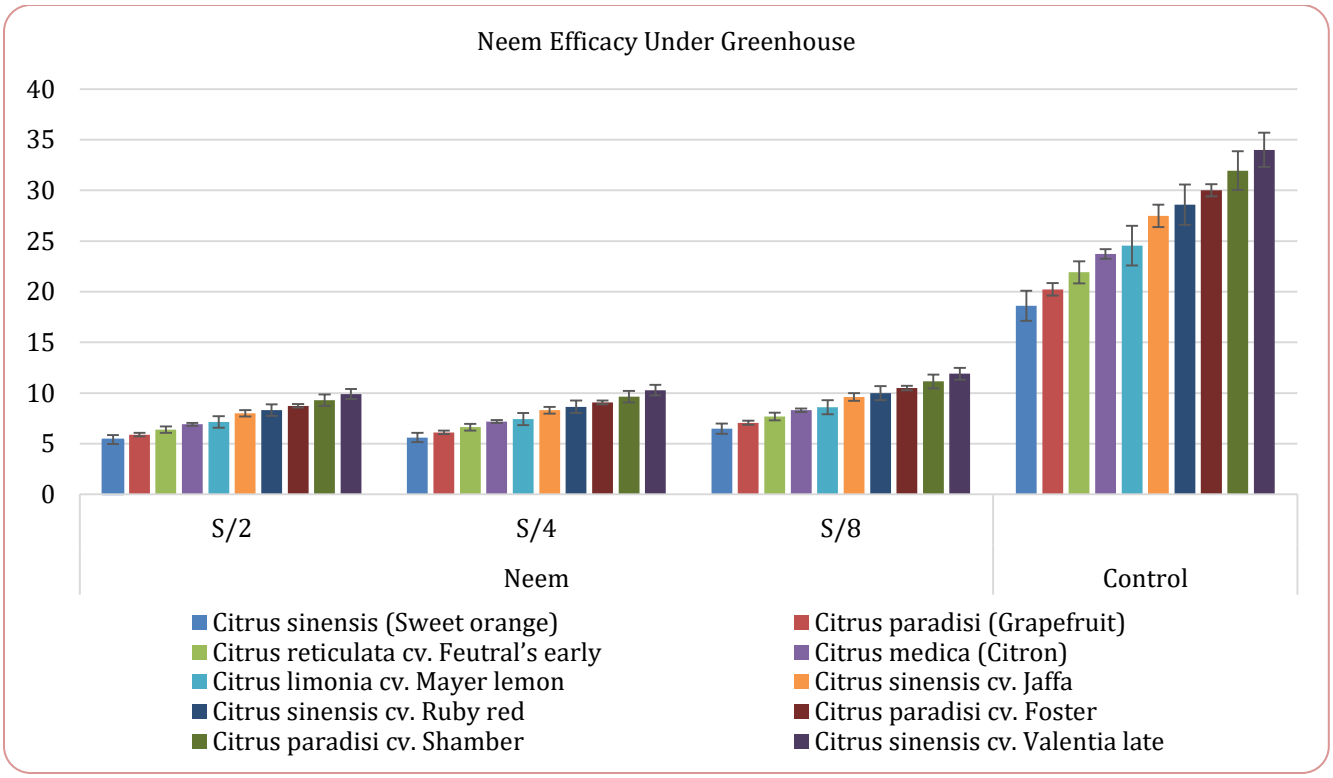

Figure 4. Percent disease severity of citrus scab in 10 citrus varieties treated with neem extract at S/2, S/4, and S/8 concentrations.

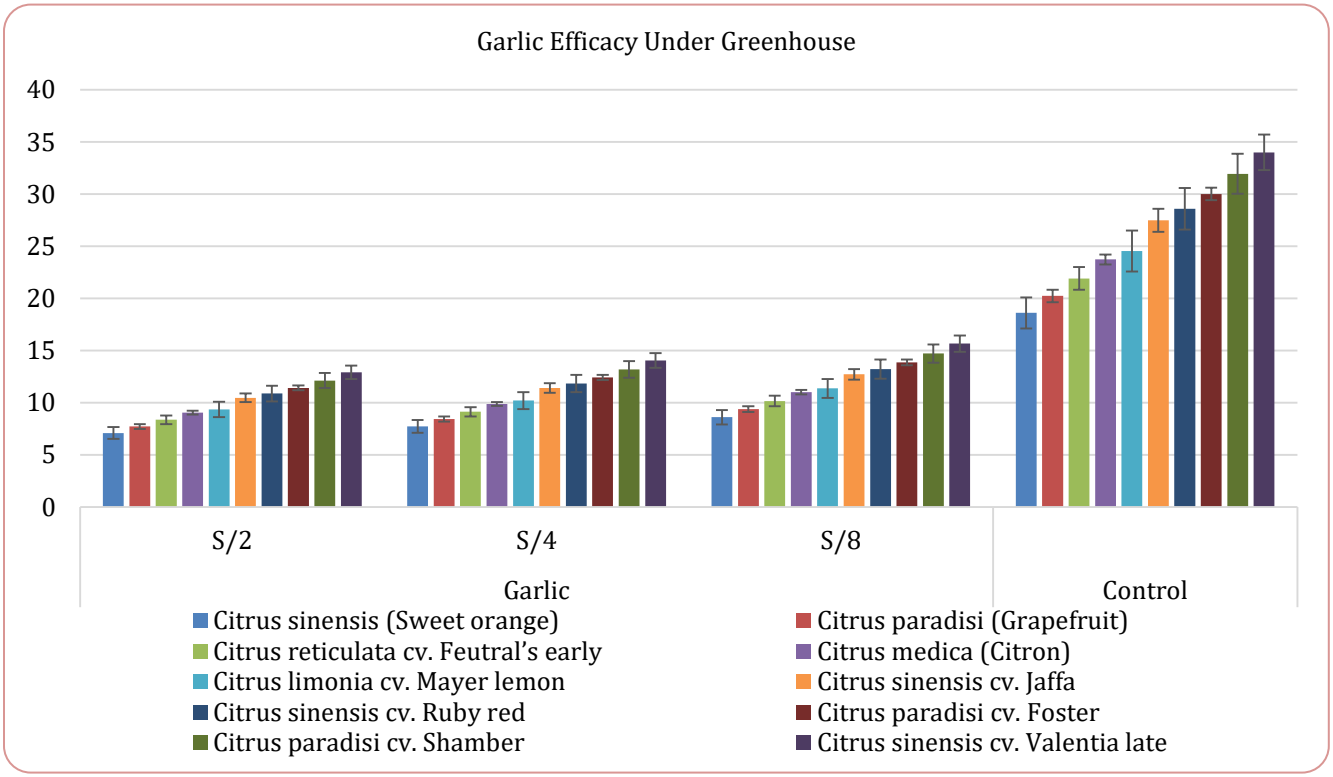

Figure 5. Percent disease severity of citrus scab in 10 citrus varieties treated with garlic extract at S/2, S/4, and S/8 concentrations.

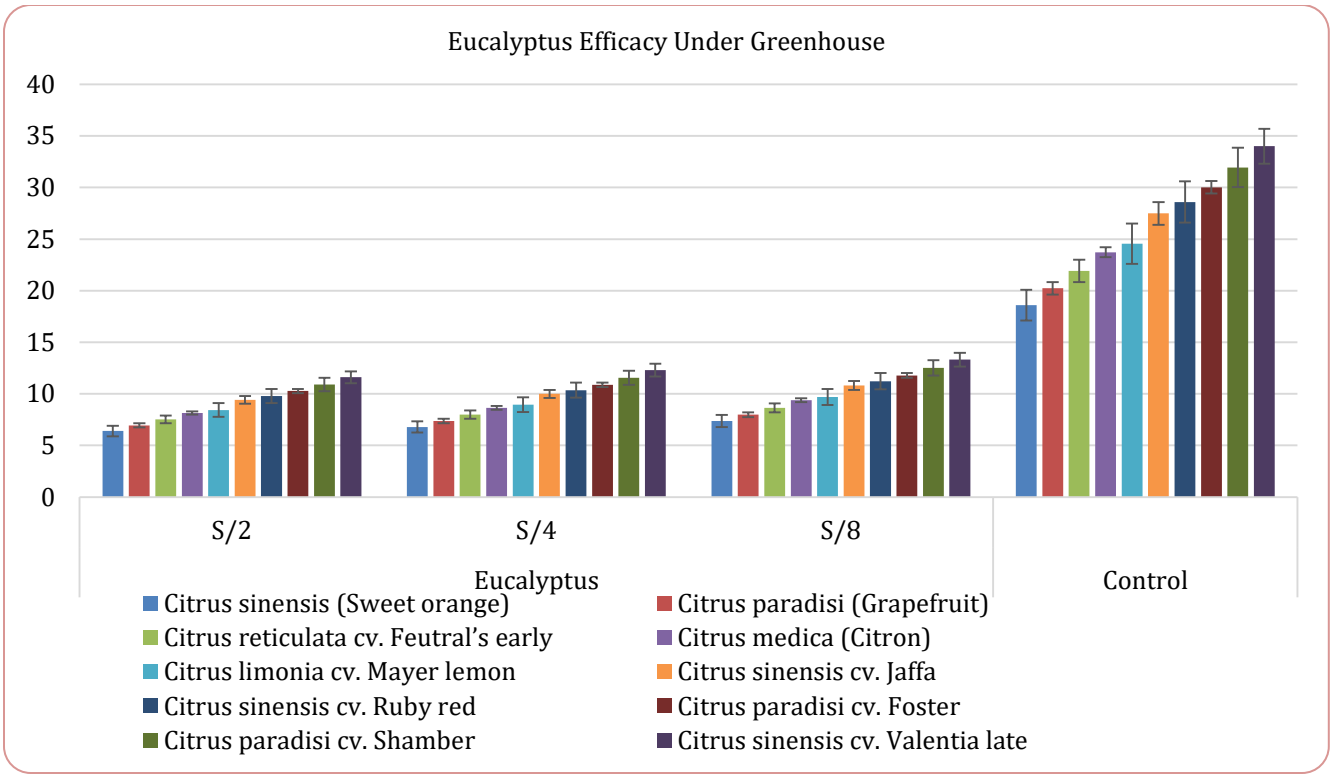

Figure 6. Percent disease severity of citrus scab in 10 citrus varieties treated with eucalyptus extract at S/2, S/4, and S/8 concentrations.

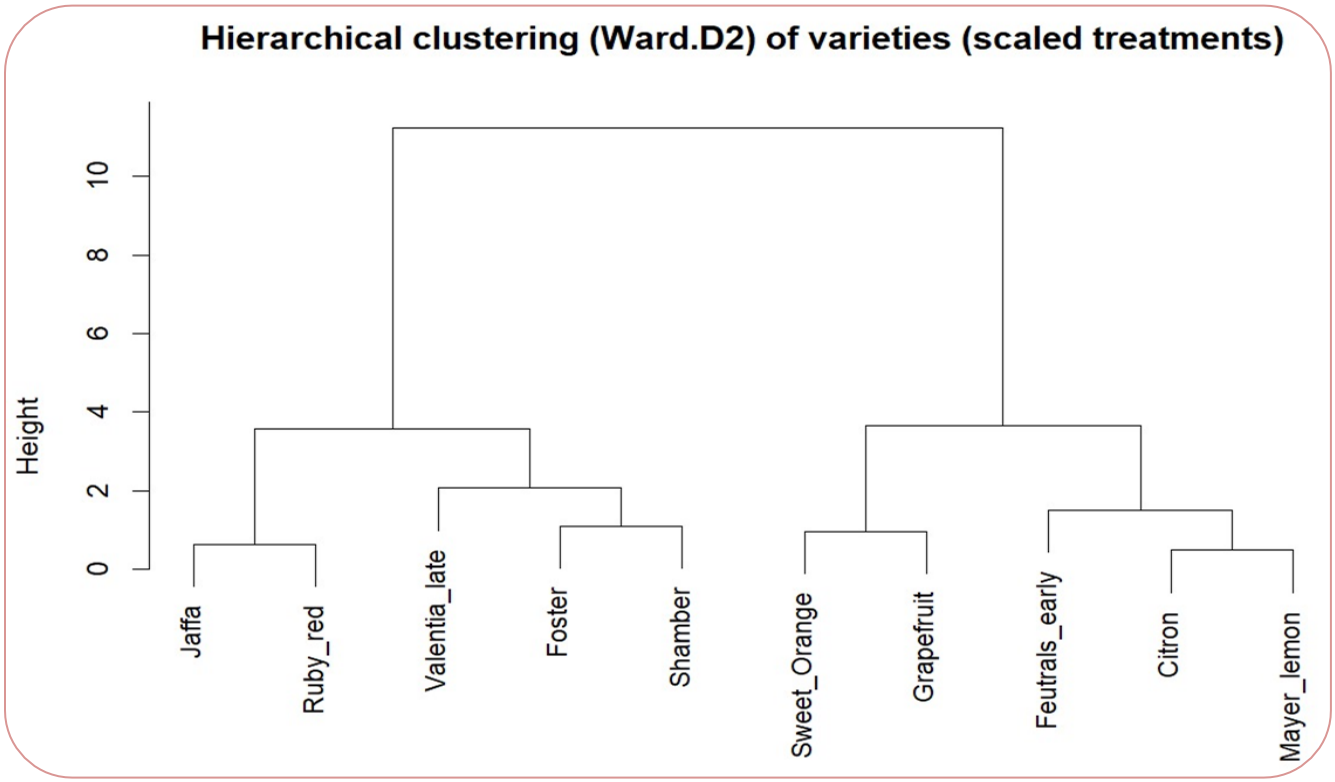

Figure 7. Hierarchical clustering of ten citrus varieties based on standardized responses to all phytoextract treatments and concentrations. Clusters correspond to similar disease response profiles.

The PCA biplot, which includes varieties together with treatment loading vectors (Figure 8), clearly explains the clustering patterns observed in the previous analysis. Along PC1, which accounted for nearly 100% of the explained variance, most of the variation was attributable to treatment effects rather than random variation, indicating that treatment responses primarily structured the model.

Neem treatments (S/2, S/4, and S/8) clustered tightly and showed strong positive loadings along the PC1 axis, indicating consistent and highly effective performance across all genotypes. Garlic treatments exhibited moderate vector lengths and directions, suggesting intermediate but stable effectiveness across varieties. In contrast, eucalyptus treatments showed the longest loading vectors, indicating greater variability and strong genotype-dependent effects.

Varieties positioned farther from the origin along the positive side of PC1, including Sweet Orange, Feutral's early, and Grapefruit, exhibited more stable and consistent responses across treatments. Conversely, susceptible varieties such as Valencia Late, Shamber, and Foster, located on the negative side of PC1 and farther from the origin, showed stronger treatment-induced shifts, indicating higher sensitivity and variability in response to the applied treatments.

A second PCA visualization (Figure 9), colored by varieties according to the overall mean percent disease reduction across treatments, further confirmed genotype-specific responses to treatment effects. Highly susceptible varieties (Shamber, Foster, and Valencia Late) exhibited the greatest reductions in disease severity, reflected by a clear shift in the PCA color gradient from cooler to warmer tones, indicating strong treatment effects relative to the control. In contrast, Citron, Mayer Lemon, and Feutral's Early showed comparatively lower percent reductions, largely attributable to their lower baseline disease severity in the control treatment rather than reduced treatment efficacy.

Overall, these multivariate analyses demonstrate that neem-based treatments provided the most consistent and effective disease suppression across genotypes, whereas eucalyptus and garlic extracts exhibited variable but generally effective responses depending on cultivar-treatment interactions.

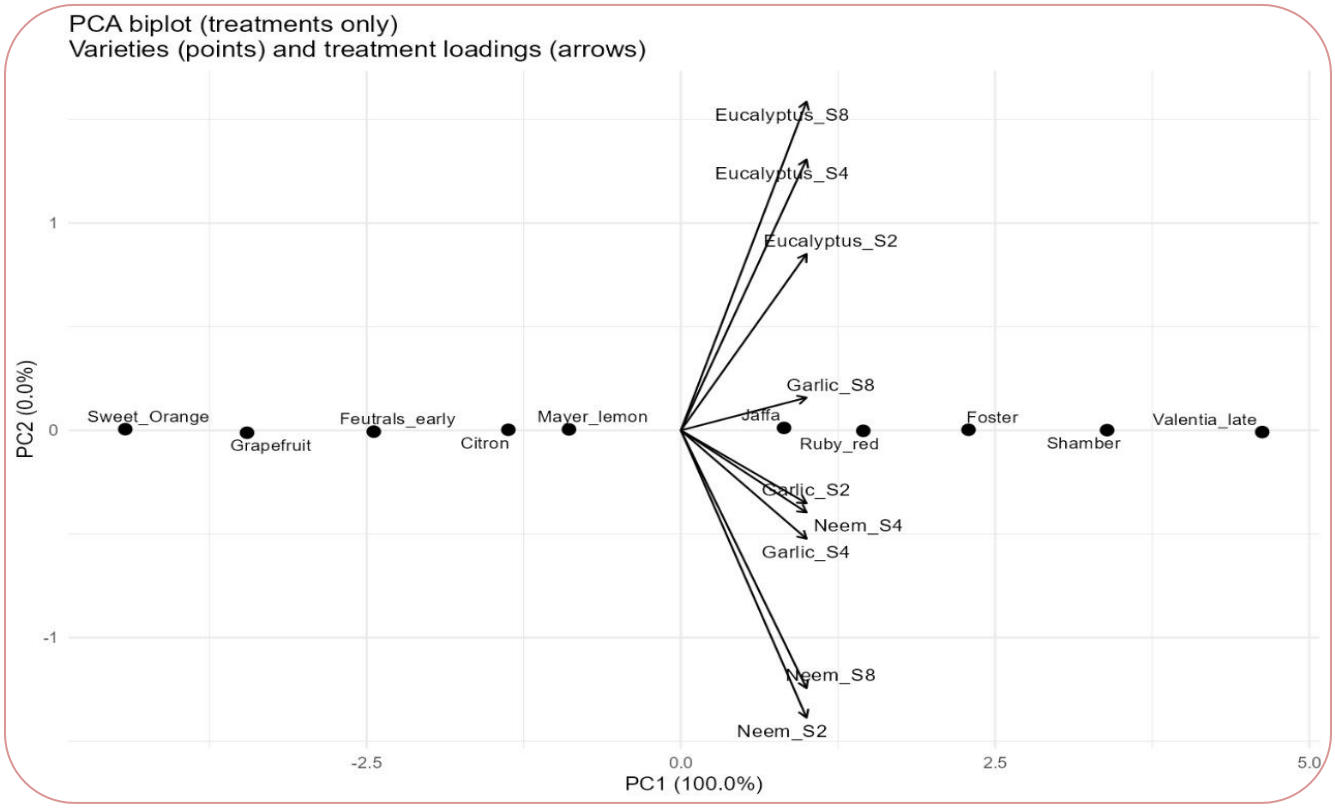

Figure 8. PCA biplot showing citrus varieties (points) and loading vectors for all treatment-concentration combinations of neem, garlic and eucalyptus.

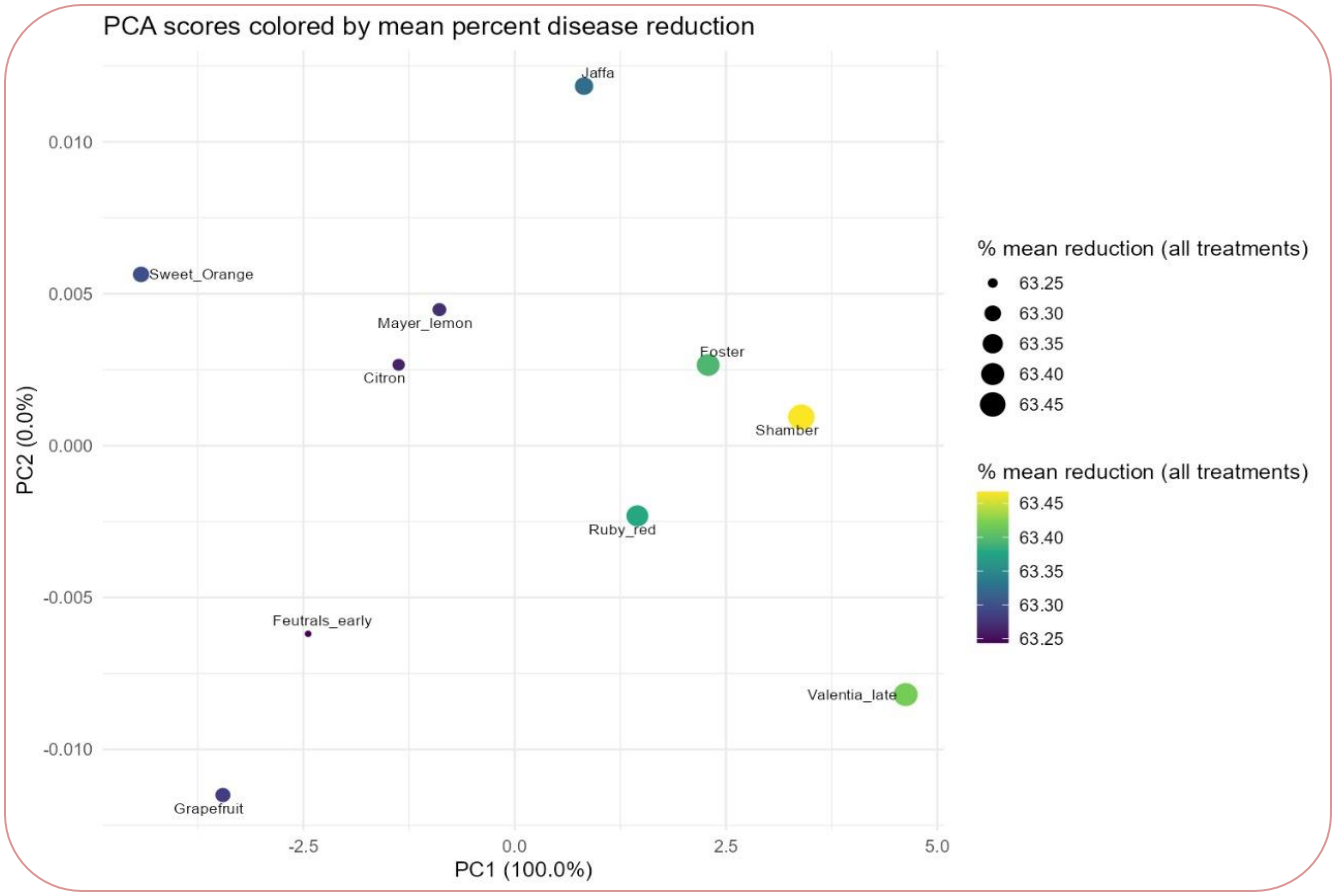

Figure 9. PCA score plot colored by mean percent disease reduction across all treatments. Warmer colors indicate stronger overall treatment responsiveness.

FTIR analysis of neem extract

The FTIR spectrum of neem extract (Figure 10) exhibited a broad O–H stretching band at approximately 3390 cm^{-1}, aliphatic C–H stretching vibrations at 2918 and 2852 cm^{-1}, a strong carbonyl (C=O) absorption band at 1732 cm^{-1} indicative of esterified compounds and limonoids, and prominent bands in the fingerprint region at 1266 and 1034 cm^{-1}, which are attributed to glycosides, flavonoids, and C–O/C–O–C functional groups. The presence of these functional groups is closely associated with the bioactive constituents of neem and supports the antifungal activity observed in the bioassays.

The FTIR profile suggests the presence of a complex mixture of phytochemicals, including phenolics, flavonoids, glycosides, and terpenoid-derived compounds. The intense broad O–H absorption band (~3390 cm^{-1}), together with the strong C–O and C–O–C bands at 1266 and 1034 cm^{-1}, indicates a high abundance of phenolic compounds and flavonoid glycosides. These compounds are known to exert antifungal effects through disruption of fungal cell walls and membranes, chelation of essential metal ions, and induction of oxidative stress within fungal cells. The prominent carbonyl band at 1732 cm^{-1} further suggests the presence of esterified triterpenoids and limonoids, including azadirachtin-like compounds, which possess well-documented antimicrobial properties and may inhibit fungal growth by interfering with cellular metabolism and membrane integrity.

The combined occurrence of phenolic, flavonoid, glycosidic, and terpenoid-related functional groups provides a plausible chemical basis for the strong antifungal activity of neem extract observed both *in vitro* and *in vivo*. Furthermore, the presence of phenolic-associated bands alongside conjugated carbonyl and aromatic absorptions around 1632 and 1600 cm^{-1} suggests a synergistic mode of action, whereby phenolic compounds may rapidly impair fungal membranes, while terpenoid and limonoid constituents exert sustained inhibitory effects on fungal metabolism and development.

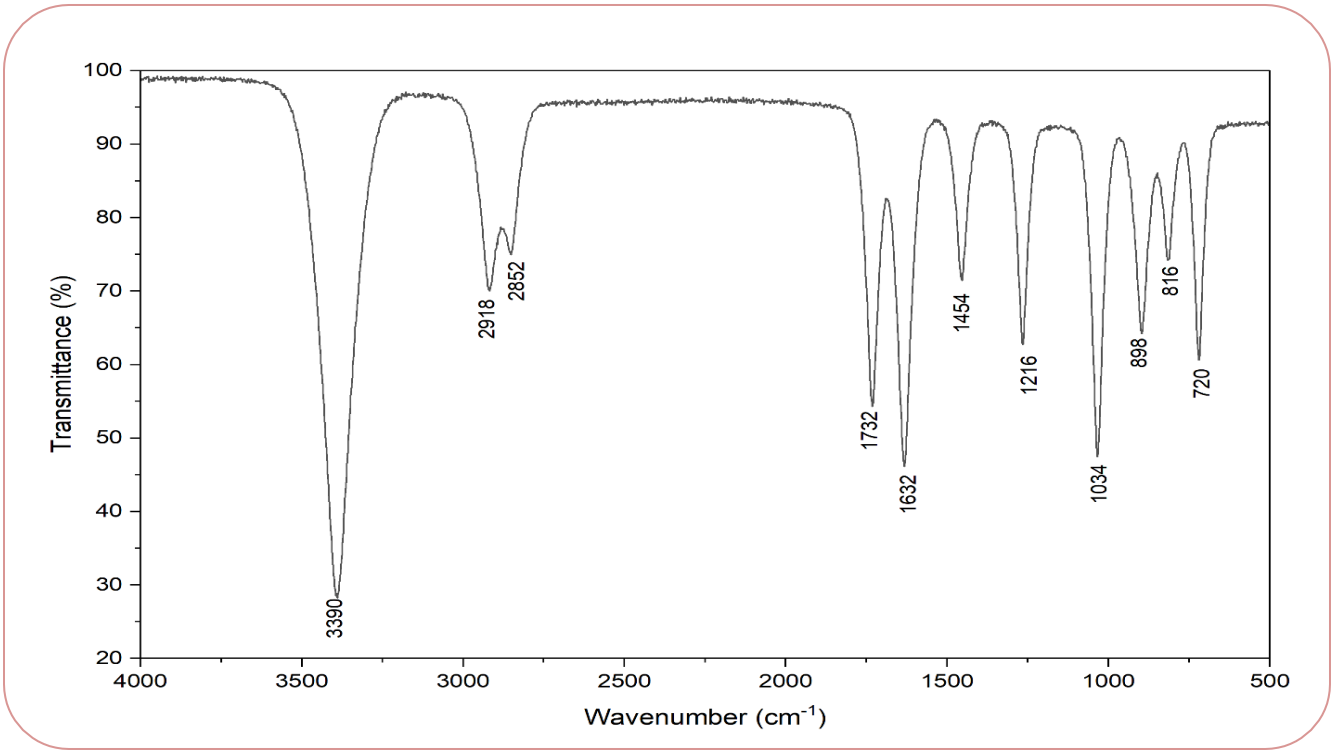

Figure 10. ATR-FTIR spectrum of neem leaf extract (4000–500 cm^{-1}). Major absorption bands are labeled with their corresponding wavenumber values.

FTIR analysis of *Eucalyptus* extract

The ATR-FTIR spectrum of the *Eucalyptus* leaf extract (Figure 11) exhibited characteristic absorption bands corresponding to O–H stretching (3476–3371 cm^{-1}), aliphatic C–H stretching (2966–2870 cm^{-1}), C=O stretching (1748 cm^{-1}), aromatic C=C stretching (1620–1508 cm^{-1}), and C–O stretching vibrations (1276–1020 cm^{-1}). These functional groups indicate the presence of phenolic compounds, terpenoids, and monoterpenes associated with strong antifungal properties.

Detailed FTIR analysis revealed a broad O–H stretching band at 3476–3371 cm^{-1}, confirming the presence of hydroxyl-containing compounds such as phenolics and alcohols. Strong aliphatic C–H stretching bands at 2966 and 2870 cm^{-1} suggested the occurrence of monoterpenes, including 1,8-cineole. A prominent carbonyl (C=O) absorption peak at 1748 cm^{-1} indicated the presence of esterified and oxygenated terpenoid compounds. Bands observed at 1620 and 1508 cm^{-1} were attributed to aromatic C=C vibrations, supporting the occurrence of polyphenolic and aromatic constituents. The characteristic C–O stretching bands at 1276, 1098, and 1020 cm^{-1} represented the diagnostic fingerprint of cineole-rich *Eucalyptus* extracts. Furthermore, several absorption bands in the fingerprint region (822–540 cm^{-1}) provided additional evidence for the presence of complex aromatic and terpenoid structures.

FTIR analysis of garlic

The ATR-FTIR spectrum of garlic extract (Figure 12) exhibited a broad absorption band at 3472–3200 cm^{-1}, corresponding to O–H and N–H stretching vibrations. Aliphatic C–H stretching bands were observed at 2992 and 2661 cm^{-1}. A distinct absorption peak at 1760 cm^{-1} indicated the presence of carbonyl (C=O) groups, characteristic of esters and other oxygenated compounds. The bands at 1674 and 1520 cm^{-1} were attributed to amide I and amide II vibrations, suggesting the presence of proteins or peptide residues. In the fingerprint region, prominent peaks at 1310, 1190, 1080, and 979 cm^{-1} corresponded to C–O, S=O, and C–S stretching vibrations, reflecting the abundance of sulfur-containing phytochemicals typical of garlic. Additional absorption bands at 860, 700, and 580 cm^{-1} were associated with aromatic and aliphatic bending vibrations. Overall, the FTIR spectrum confirmed the presence of phenolic compounds, organosulfur constituents, and carbonyl-containing metabolites, which may contribute to the antifungal activity of the garlic extract.

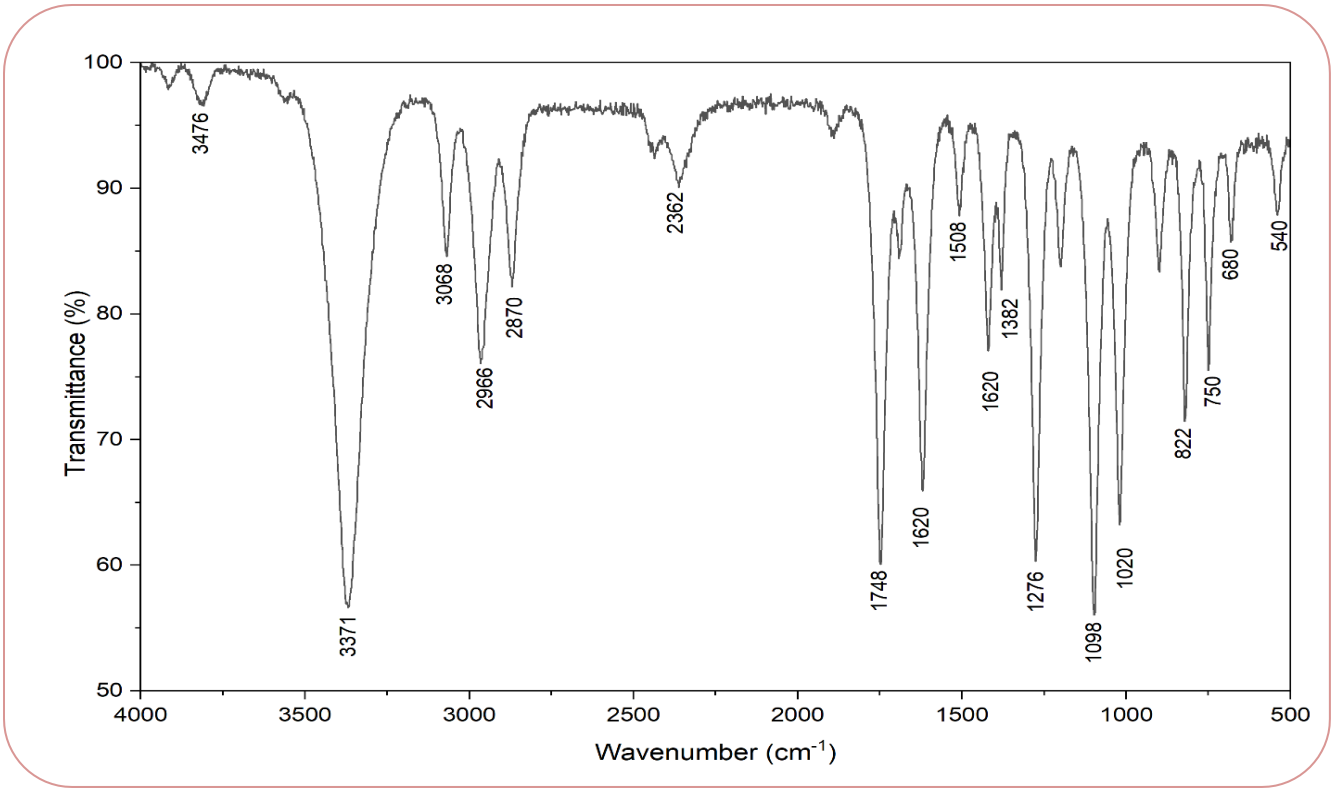

Figure 11. ATR-FTIR spectrum of *Eucalyptus* leaf extract (4000–500 cm^{-1}), showing the major absorption bands labeled with their corresponding wavenumber values.

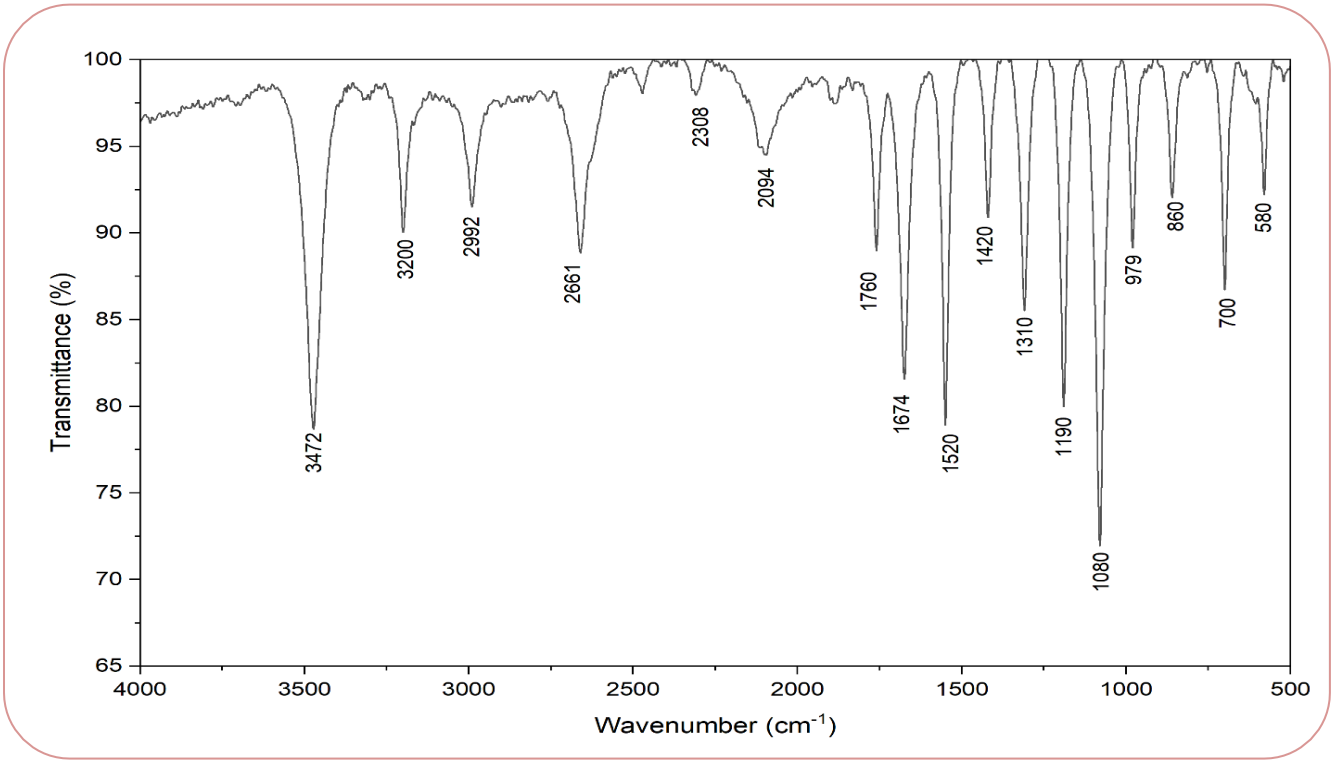

Figure 12. FTIR spectrum of garlic extract showing characteristic O–H, C–H, C=O, amide, and sulfur-containing functional groups associated with bioactive compounds exhibiting antifungal activity.

Discussion

The present study provides a comprehensive evaluation of the antifungal efficacy of ten botanical extracts against *E. fawcettii*, integrating *in vitro* mycelial growth inhibition, greenhouse assessments across ten susceptible citrus cultivars, and phytochemical characterization through FTIR spectroscopy. Although the antifungal activity of botanical extracts against foliar pathogens has been widely reported (Nxumalo et al., 2021), this study offers novel understandings by quantifying cultivar-specific disease suppression, assessing concentration-dependent responses across multiple citrus genotypes, and associating antifungal efficacy with functional groups identified through FTIR analysis. The observed genotype-specific responses highlight the importance of host-extract interactions and provide a basis for selecting promising botanical extracts for future field evaluation and the development of standardized plant-based disease management strategies.

The patterns of mycelial inhibition observed in the present study are consistent with previous reports demonstrating the broad-spectrum antifungal properties of neem, eucalyptus, and garlic extracts (Nxumalo et al., 2021). The antifungal activity of neem is primarily attributed to limonoids such as azadirachtin and nimbin, which disrupt fungal cell membranes and inhibit ergosterol biosynthesis, an essential component of fungal cell membranes (Deresa and Diriba, 2023). Likewise, eucalyptol from eucalyptus and allicin from garlic exert fungitoxic effects by inducing reactive oxygen species (ROS), inhibiting key fungal enzymes, and compromising membrane integrity (Shin et al., 2021; Upadhayay et al., 2024). Similar levels of inhibition have been reported against other phytopathogenic fungi. For example, neem extract suppressed *Colletotrichum boninense* growth by 65-75% (Kushaha et al., 2024), whereas garlic extract inhibited *Sclerotinia sclerotiorum* by approximately 58% (Upadhayay et al., 2024). Furthermore, the moderate antifungal activity observed for cinnamon and turmeric extracts is in agreement with previous findings suggesting that cinnamaldehyde- and curcumin-rich extracts are often more effective when used as components of integrated or synergistic treatments (Kushaha et al., 2024).

Greenhouse evaluations demonstrated that the three most effective extracts, neem, eucalyptus, and garlic, significantly reduced citrus scab severity across all tested cultivars. Disease severity in susceptible and highly susceptible cultivars (18.60-34.00%) was reduced to levels corresponding to resistant or tolerant reactions. Neem extract consistently produced the greatest reduction, followed by eucalyptus and garlic. A particularly noteworthy finding was the marked reduction in disease severity in the highly susceptible cultivar *Citrus sinensis* cv. Valencia Late, where disease severity declined from 34.00% to 9.90%. The differential response among cultivars reflects the complexity of host-pathogen interactions and may be associated with variations in phytohormonal regulation, defense activation, and disease progression among citrus species and cultivars (Shin et al., 2021). Moreover, the greenhouse conditions closely simulated the environmental conditions favorable for citrus scab development, including prolonged leaf wetness and splash-mediated pathogen dispersal, thereby enhancing the biological relevance of the observed disease suppression (Dewdney, 2025).

The efficacy of botanical extracts is likely attributable to multiple complementary mechanisms. In addition to direct antifungal effects through membrane disruption and metabolic inhibition, plant extracts may enhance host defense responses and physiological resilience. Improved uptake of essential nutrients such as calcium and zinc has been associated with strengthened cell wall integrity and enhanced resistance against fungal infection (Shabbir et al., 2022; Tripathi et al., 2022). Such multi-target modes of action may provide a valuable alternative to synthetic fungicides, particularly in addressing the growing problem of fungicide resistance in *E. fawcettii*, including resistance to strobilurin fungicides (Masheti et al., 2024; Dewdney, 2025). In contrast, many conventional fungicides act on a single molecular target, increasing the risk of resistance development (Islam et al., 2024).

FTIR spectroscopy further confirmed the presence of bioactive functional groups associated with antifungal activity. Neem extract exhibited prominent O–H, C–O/C–O–C, and ester carbonyl (C=O) absorption bands, indicating the presence of phenolics, glycosides, and triterpenoid limonoids (Mohanasundaram and Saral, 2023). Eucalyptus extract displayed characteristic O–H, aliphatic C–H, and C–O bands associated with monoterpenes such as 1,8-cineole, compounds known to alter fungal membrane permeability and induce

oxidative stress (Čmiková et al., 2023). Garlic extract exhibited distinct sulfur-containing functional groups (S=O and C–S), corresponding to organosulfur compounds such as allicin and ajoene, which interfere with fungal respiration, redox homeostasis, and mitochondrial activity (Kobacy et al., 2025). These findings are consistent with recent FTIR-based phytochemical studies identifying phenolic hydroxyl groups, terpenoid carbonyls, and sulfur-containing compounds as key biomarkers of antifungal potential in botanical extracts (Patle et al., 2020; Rohaeti et al., 2021; Shaheen et al., 2022). Collectively, the FTIR results provide mechanistic support for the strong antifungal activity observed in both laboratory and greenhouse experiments.

Despite their promising performance, the efficacy of botanical extracts under greenhouse conditions may not always translate directly to field environments. Variations in phytochemical composition resulting from differences in plant genotype, geographic origin, seasonal conditions, and extraction procedures can significantly influence biological activity (Pries et al., 2023; Batista et al., 2024). Furthermore, factors such as photodegradation, limited residual activity, and potential phytotoxicity require careful assessment before large-scale field application. Therefore, these extracts should currently be regarded as promising candidates for further development rather than direct replacements for registered fungicides (Wylie and Merrell, 2022). Future studies should focus on multi-location field evaluations, detailed phytochemical profiling using GC–MS and LC–MS/MS, bioassay-guided fractionation, nanoformulation approaches to improve stability and delivery, and molecular investigations of plant defense activation and pathogen inhibition pathways (Van Heerden et al., 2024). The integration of such botanical extracts into integrated pest management (IPM) programs could substantially reduce citrus scab severity in major citrus-producing regions, including Pakistan, where citrus contributes approximately 2.48% to the agricultural GDP and citrus scab may cause annual yield losses of 20-30% (Dwiastuti et al., 2021; Siddique et al., 2024).

Conclusion

The present study identified neem, eucalyptus, and garlic extracts as effective and environmentally sustainable alternatives for managing citrus scab caused by *Elsinoë fawcettii*. Under *in vitro* conditions, neem extract exhibited the highest antifungal activity, achieving 70.70% mycelial growth inhibition after nine days at the highest concentration, followed by eucalyptus (65.65%) and garlic (61.91%). Greenhouse evaluations across ten susceptible citrus cultivars demonstrated that these extracts reduced disease severity by 55-75% compared with untreated controls, transforming susceptible and highly susceptible cultivars into resistant or tolerant categories. Neem consistently provided the greatest level of disease suppression. The antifungal activity of these extracts is associated with bioactive compounds capable of disrupting fungal cell membranes, inhibiting ergosterol biosynthesis, inducing oxidative stress, and enhancing host defense mechanisms. Given the increasing challenge of fungicide resistance, botanical extracts offer promising alternatives for sustainable citrus disease management. Nevertheless, field validation, phytochemical standardization, and formulation optimization are required before their large-scale adoption within integrated pest management programs.

Limitations and future prospects

A major limitation of the present study is the absence of detailed phytochemical characterization and quantification beyond FTIR analysis. Although FTIR successfully identified functional groups associated with antifungal activity, it does not provide precise information regarding the identity and concentration of individual bioactive compounds. Previous studies have shown that phytochemical composition varies considerably with plant species, geographical origin, plant part, environmental conditions, and extraction methodology, thereby influencing biological activity (Wylie and Merrell, 2022). Future research should therefore incorporate comprehensive phytochemical profiling using GC–MS for volatile and essential oil fractions and LC–MS/MS or HPLC-DAD/ESI-MS for non-volatile compounds. Bioassay-guided fractionation would further facilitate the identification of specific metabolites responsible for antifungal activity. Recent studies on neem extracts and essential oils have demonstrated the effectiveness of combining chromatographic and spectrometric techniques to identify limonoids, terpenoids, and other antifungal constituents responsible for disease suppression (Schuch et al., 2024). Such investigations would improve

the mechanistic understanding of botanical fungicides and support the development of standardized, commercially viable formulations for citrus scab management.

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Authors' Contributions

UA conducted the experimental work, collected and organized the data, and contributed to manuscript preparation. STS conceived, designed, and supervised the study. MA provided technical support and performed the data analysis. MAK critically revised, finalized, and proofread the manuscript. All authors reviewed and approved the final version of the manuscript.

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Conflict of Interest

The authors declare no conflict of interest.

Sustainable Development Goals Targeted

SDG 2: Zero Hunger
SDG 12: Responsible Consumption and Production
SDG 15: Life on Land

Policy Addressed

The study strongly supports a shift toward green plant protection strategies that align with sustainable agriculture goals, environmental safety, and long-term citrus industry resilience.

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