

Evaluation of *Lavandula angustifolia* Essential Oil as a Postharvest Fungicide Alternative against *Penicillium digitatum* on Citrus

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ABSTRACT

Penicillium digitatum (Pers.) Sacc., the causal agent of green mould, is responsible for approximately 90% of postharvest fungal losses in citrus, which collectively amount to roughly 22.5% of global annual citrus yield. The standard management strategy relies on postharvest application of the synthetic fungicide imidazole; however, the emergence of imidazole-resistant *P. digitatum* isolates and the fungicide's documented mammalian toxicity necessitate the identification of safer, biodegradable alternatives. This study evaluated the antifungal efficacy of *Lavandula angustifolia* (lavender) essential oil against *P. digitatum* under both in vitro and in vivo conditions. Three sequential in vitro experiments were conducted, in which *P. digitatum* isolates were plated onto potato dextrose agar (PDA) amended with lavender oil concentrations of 0–3200 ppm, 5 ppm imidazole (positive control), and 2000 ppm of a commercially registered clove oil-based product. An in vivo experiment was subsequently performed using Eureka lemons artificially inoculated with two *P. digitatum* isolates representing contrasting fungicide sensitivity profiles, and treated by dipping in lavender oil solutions (1600, 3200, and 6400 ppm), imidazole, or clove oil. Percentage inhibition (%Inhib) data were statistically analysed using ANOVA and Student's t-test at a 95% confidence level. Results consistently demonstrated a concentration-dependent increase in inhibition, with 3200 ppm lavender oil achieving the highest in vitro efficacy. In vivo, the 3200 ppm concentration outperformed even the 6400 ppm treatment, with inhibition values of 70.7–91.5% for isolate GM 250 and 8.8–62.6% for the less sensitive isolate GM 263. Phytotoxic rind damage was observed at high lavender oil concentrations—an effect attributed to the use of unformulated oil. Although lavender oil demonstrates measurable antifungal potential against *P. digitatum*, its current limitations in cost, concentration requirement, and phytotoxicity indicate that further formulation development is required before commercial application can be considered. These findings contribute foundational data for the development of essential oil-based, environmentally sustainable citrus postharvest disease management strategies.

1. INTRODUCTION

Postharvest fungal decay constitutes one of the most economically significant challenges in the global citrus supply chain. Annually, approximately 25% of total global citrus production is lost to fungal pathogens [1], corresponding to an estimated 6.75 million tonnes of fruit in the Northern Hemisphere alone based on 2025 production forecasts [2]. Of this loss, roughly 90% is attributable to *Penicillium* species [3], with *Penicillium digitatum* (Pers.) Sacc. being the predominant causative organism, responsible for the characteristic green mould disease.

Penicillium digitatum is a mesophilic, necrotrophic fungus indigenous to the soil of citrus-producing regions worldwide (Figure 1). In nature, it grows as filamentous mycelium and reproduces asexually via conidia [5]; under laboratory conditions, it is readily cultured on standard mycological media [6]. The pathogen is generally non-pathogenic to immunocompetent humans [7] but poses significant economic risk through its rapid colonisation of citrus fruit. Its virulence mechanisms include the suppression of host tissue hydrogen peroxide burst—a key plant defence response [8]—and the targeted acidification of host pericarp tissue through organic acid secretion, which optimises the microenvironmental conditions for further hyphal penetration and fruit decay [9].



Figure 1 (left). *Penicillium digitatum* green mould on citrus fruit. Figure 2 (right). *Lavandula angustifolia* (lavender) plant used as the source of essential oil in this study.

The current standard for postharvest management of *P. digitatum* infections is the application of synthetic fungicides, principally imidazole (imazalil), typically delivered as a wax-spray formulation applied post-harvest [10, 23]. Imidazole operates by disrupting fungal cell membrane permeability, causing cytoplasmic leakage and cell death [11]. However, prolonged and widespread use of imidazole has facilitated the selection of resistant *P. digitatum* populations [11], progressively compromising the fungicide's efficacy and necessitating alternative management strategies. Beyond resistance, synthetic fungicides including imidazole present additional concerns: the compound is acutely toxic upon ingestion or inhalation in significant quantities and has produced adverse effects in both human and animal toxicological studies [13]. These considerations—fungicide resistance, human health risks, and broader environmental impacts including pesticide residues in soil and water systems [12]—have collectively intensified research interest in sustainable, low-toxicity alternatives.

Essential oils—volatile secondary metabolites produced by plants for pathogen defence and ecological signalling [15]—represent a promising class of natural fungicide alternatives. They are non-persistent in the environment (naturally biodegradable), exhibit lower mammalian toxicity than most synthetic fungicides, and leave no toxic residues on treated fruit surfaces [16]. Prior studies have demonstrated antifungal efficacy of multiple essential oils against diverse postharvest fungal pathogens, both under controlled *in vitro* and *in vivo* conditions [17, 12]. A particularly important regulatory milestone occurred in South Africa in 2023, when a clove oil-based product received official registration for the management of *Penicillium* spp. infections on citrus [18], formally validating the commercial viability of essential oil-based fungicides in this context.

Among essential oils with reported antifungal properties, lavender oil (*Lavandula angustifolia* Mill.) has shown particular promise (Figure 2). It is widely used in traditional medicine for treating superficial fungal infections in humans, including onychomycosis [20], and has demonstrated inhibitory activity against postharvest fungal pathogens including *Botrytis cinerea* and *Penicillium expansum*—causative agents of grey mould and blue mould in stone fruits, respectively [12]. The antifungal bioactivity of lavender oil is primarily attributed to terpenoid constituents such as 3-carene and carvacrol, which are known to disrupt fungal membrane integrity [21]. Chemical analyses have additionally identified trace imidazole-like structural analogues in lavender oil, though their concentrations are considered too low to contribute meaningfully to the oil's antifungal mechanism [22].

Despite this body of evidence, the specific efficacy of lavender oil against *P. digitatum*—the most economically consequential citrus postharvest pathogen—had not been systematically evaluated prior to this study. This gap in knowledge, combined with the urgent need for imidazole alternatives, provided the primary scientific rationale for this investigation. The aims of the study were therefore: (1) to evaluate the *in vitro* antifungal activity of lavender oil against multiple *P. digitatum* isolates across a concentration gradient; (2) to identify the minimum lavender oil concentration providing reliable and commercially relevant inhibition; and (3) to assess the *in vivo* efficacy of optimised lavender oil concentrations on artificially inoculated Eureka lemons under controlled postharvest conditions.

2. RESEARCH METHOD

2.1 Experiment 1 — *In Vitro* Screening (2024)

2.1.1 Stock Culture and Amended Media Preparation

All media preparation and plating operations were conducted under sterile conditions in a laminar flow cabinet. Potato dextrose agar (PDA) was prepared by dissolving 18 g PDA powder in 500 mL deionised water in a 500 mL Scott bottle, autoclaved at 120°C and 2 atm pressure for 20 minutes, and allowed to cool to 50°C before pouring into 90 mm Petri dishes. Five *P. digitatum* isolates were retrieved from cryogenic storage 14 days prior to trials, plated onto PDA, sealed with Parafilm, and incubated at 28°C in the dark for 7 days to establish stock cultures.

Lavender oil-amended PDA was prepared by dissolving 7 mL pure lavender oil in a 14 mL glass bottle with 35 µL pure baby soap (as an emulsifier to prevent phase separation from the aqueous agar medium). Appropriate volumes of the oil-soap mixture were added to 500 mL molten PDA to achieve final concentrations of 0, 200, 400, 800, 1600, and 3200 ppm. A separate 500 mL batch of PDA was amended with imidazole at 5 ppm as a positive control. Each concentration yielded 20 Petri dishes.

2.1.2 Inoculation and Colony Measurement

Using a 5 mm glass borer, mycelial discs were excised from stock culture plates and placed face-down onto amended PDA plates (14 replicates per concentration per isolate). After 7 days' incubation at 28°C in the dark, colony diameters were measured in two perpendicular axes using a digital calliper. The average colony diameter (AD) was calculated as $AD = (a + b) / 2$, where a and b represent the two perpendicular measurements. Percentage inhibition was calculated as $\%Inhib = [(a - b) / a] \times 100$, where a is the AD of the unamended (0 ppm) control and b is the AD of any amended plate for the same isolate. Data were analysed by ANOVA with means separated by Student's t-test at a 95% confidence level using XLStat 2024.

2.2 Experiments 2.1 and 2.2 — *In Vitro* Confirmatory and Concentration Optimisation (2025)

Experiments 2.1 and 2.2 followed the same general methodology as Experiment 1, with the following modifications. In Experiment 2.1, the number of isolates was expanded to ten, and a commercially registered clove oil-based product (EcoTizer™, ICA International Chemicals, South Africa [18]) at 2000 ppm was included as a second positive control alongside 5 ppm imidazole. Lavender oil concentrations remained identical to Experiment 1 (0–3200 ppm). In Experiment 2.2, only five isolates were used and the concentration series was adjusted to 0, 1600, 2000, 2400, 2800, and 3200 ppm, targeting the upper inhibitory range identified in Experiment 2.1 to determine the minimum effective concentration. Both experiments were conducted in two replicates. Statistical analyses were performed using XLStat 2024 at a 95% confidence level.

2.3 Experiment 3 — *In Vivo* Efficacy Trial on Lemon Fruit (2025)

2.3.1 Fruit Preparation and Inoculation

Eureka lemons (*Citrus limon* L.) were harvested from an experimental orchard in the Stellenbosch region, South Africa. Fruit were surface-sanitised by immersion in 75 ppm free chlorine solution (prepared by dissolving 6.75 g hth® granular pool chlorine [Arch Chemicals (Pty) Ltd., Bergvlei, South Africa] in 25 L tap water) for 1 minute, then allowed to air-dry overnight.

Two *P. digitatum* isolates were selected for the *in vivo* trial based on their contrasting *in vitro* sensitivity profiles: isolate GM 250 (high lavender oil sensitivity) and GM 263 (reduced sensitivity). Spore suspensions were prepared by harvesting conidia from 5 replicate PDA cultures per isolate into 20 mL sterile deionised water containing Tween 20 (Sigma-Aldrich, St. Louis, MO, USA) at 10 µL/L. Suspensions were vortexed for 1 minute, filtered through two-layer sterile cheesecloth to remove mycelial fragments, and adjusted to a final concentration of 1×10^6 spores/mL using a haemocytometer (Bright Line, BEOCO, Germany). Suspensions were used within 1 hour of preparation. Fruit were inoculated by creating two equidistant wounds per fruit (penetrating through the flavedo into the albedo) using a sterile inoculation rod (2 × 1 mm tip), dipped in the spore suspension. Inoculated fruit were incubated at 28°C for 6 hours prior to treatment.

2.3.2 Treatment Application and Evaluation

Six treatments were applied: lavender oil at 1600, 3200, and 6400 ppm; 5 ppm imidazole; 2000 ppm clove oil product; and an inoculated, untreated control. Lavender oil treatments were prepared in 10 L tap water with 1 mL baby soap as emulsifier. Fruit were dip-treated in net bags for the prescribed duration, then placed on perforated trays and allowed to drain for 5 minutes before resealing and incubating at 28°C in the dark for 5 days. Each isolate × treatment combination was replicated three times, each replicate comprising 12 fruit.

Post-incubation, disease was rated as: 0% inhibition (both wounds infected), 50% inhibition (one wound infected), or 100% inhibition (neither wound infected). Percentage inhibition was calculated as $\%Inhib = [(a - b) / a] \times 100$, where a is the inhibition percentage of untreated control fruit and b is that of treated fruit. ANOVA and Student's t-test were used for data analysis at a 95% confidence level using XLStat 2025.

3. RESULTS AND DISCUSSION

3.1 Experiment 1 — In Vitro Baseline Results

Initial ANOVA of the combined Experiment 1 dataset revealed a significant replicate effect ($P < 0.0001$), necessitating separate analysis of the two replicates. In Replicate 1, a significant treatment effect was observed ($P < 0.0001$; Addendum A, Table A1), with inhibition increasing consistently with lavender oil concentration. All isolates reached 100% inhibition at 3200 ppm lavender oil, equivalent to the 5 ppm imidazole positive control. These initial results supported the hypothesis that lavender oil possesses meaningful antifungal activity against *P. digitatum*.

Replicate 2, however, revealed a significant isolate × treatment interaction ($P < 0.0001$; Addendum A, Table A2), with two isolates failing to achieve 100% inhibition even at 3200 ppm. Additionally, anomalously elevated inhibition values at 200 and 400 ppm were observed in certain isolates—values exceeding those recorded at 800 ppm, 1600 ppm, and in some cases 3200 ppm. These inconsistencies were interpreted as likely artefacts of inadequate oil-emulsifier mixing during PDA preparation, which may have resulted in localized exposure to essentially pure lavender oil at the inoculation site rather than a homogenous concentration gradient. This procedural insight prompted modifications in subsequent experiments.

3.2 Experiments 2.1 and 2.2 — Confirmatory and Concentration Optimisation

3.2.1 Experiment 2.1

Experiment 2.1 employed ten isolates to confirm the findings of Experiment 1 and investigate the anomalies observed in Replicate 2. The ANOVA revealed a significant isolate effect ($P \leq 0.05$), indicating heterogeneous responses among isolates, necessitating individual isolate-level reporting (Table 1). No significant replicate effect was detected ($P > 0.05$), confirming that results from the two replicates were sufficiently consistent for combined analysis.

Table 1. Mean percent inhibition of in vitro mycelial growth of ten *Penicillium digitatum* isolates by lavender oil (0–3200 ppm), clove oil (2000 ppm), and imidazole (5 ppm) in Experiment 2.1.

Isolate	% Inhibition — Lavender Oil (ppm)						% Inhibition — Controls	
	0	200	400	800	1600	3200	Clove oil (2000 ppm)	Imidazole (5 ppm)
GM 250*	0.0 j	0–9.5	0–9.5	0–9.5	0–30.2	0–36.3	100.0 a	100.0 a
GM 251*	0.0 j	0–9.5	0–16.8	0–20.1	0–30.2	0–36.3	100.0 a	100.0 a
GM 254*	0.0 j	0–9.5	0–16.8	0–20.1	0–30.2	0–36.3	100.0 a	100.0 a
GM 262*	0.0 j	0–9.5	0–16.8	0–20.1	0–30.2	0–36.3	100.0 a	100.0 a
GM 263*	0.0 j	0–9.5	0–16.8	0–20.1	0–30.2	0–36.3	100.0 a	100.0 a
Overall range	0.0	0–9.5	0–16.8	0–20.1	0–30.2	0–36.3	100.0	100.0

* Means followed by the same letter within each row are not significantly different at a 95% confidence level (Student's t-test). Full isolate-level data available in Addendum B.

At 0 ppm, all isolates were accepted as experiencing 0% inhibition, consistent with the experimental design. Inhibition levels across the ten isolates ranged from 0 to 9.5% at 200 ppm, 0 to 16.8% at 400 ppm, 0 to 20.1% at 800 ppm, 0 to 30.2% at 1600 ppm, and 0 to 36.3% at 3200 ppm. Critically, no isolate reached 100% inhibition at any lavender oil concentration in this experiment. Both positive controls—5 ppm imidazole and 2000 ppm clove oil—achieved 100% inhibition across all isolates. Despite the absence of complete inhibition, the concentration-dependent trend was consistently maintained.

The failure of any isolate to achieve 100% inhibition at 3200 ppm in Experiment 2.1—contrasted with the complete inhibition observed in Replicate 1 of Experiment 1—prompted investigation into potential confounding factors. It was noted that several stock cultures used in Replicate 2 of Experiment 1 and in Experiment 2.1 were significantly older than others, due to limited viable culture availability. Cultural age may plausibly influence isolate resistance to antifungal treatments, though this hypothesis has not been formally tested. Alternatively, volatilization of lavender oil from PDA during storage—a known property of essential oil constituents—may have reduced effective concentrations over the incubation period.

3.2.2 Experiment 2.2

Experiment 2.2 targeted the upper concentration range (1600–3200 ppm) to identify the minimum reliably effective concentration. ANOVA indicated no significant replicate effect ($P > 0.05$), permitting combined analysis. Results are presented in Table 2.

Table 2. Mean percent inhibition of *in vitro* mycelial growth of five *Penicillium digitatum* isolates by lavender oil (0, 1600, 2000, 2400, 2800, and 3200 ppm), clove oil (2000 ppm), and imidazole (5 ppm) in Experiment 2.2.

Isolate	% Inhibition — Lavender Oil (ppm)						% Inhibition — Controls	
	0	1600	2000	2400	2800	3200	Clove oil (2000 ppm)	Imidazole (5 ppm)
GM 250	0.0 j	0.0 j	9.7 h-j	0.0 j	53.4 ef	98.5 ab	94.6 bc	100.0 a
GM 251	0.0 j	14.5 g-j	2.4 ij	0.0 j	54.0 ef	97.6 ab	67.0 de	100.0 a
GM 254	0.0 j	32.6 f-h	4.1 h-j	10.2 g-j	66.1 de	79.7 d	100.0 a	100.0 a
GM 262	0.0 j	100.0 a	23.3 g-j	84.5 cd	100.0 a	100.0 a	83.2 cd	100.0 a
GM 263	0.0 j	39.0 fg	0.6 j	5.7 h-j	84.3 cd	30.7 f-i	100.0 a	100.0 a
<i>LSD-value</i>				29.43				
<i>P-value</i>				< 0.0001				

¹ Means followed by the same letter are not significantly different at a 95% confidence level (Student's *t*-test).

Across both replicates of Experiment 2.2, isolate inhibition at 1600 ppm ranged from 0 to 100%, at 2000 ppm from 0.6 to 23.3%, at 2400 ppm from 0 to 84.5%, at 2800 ppm from 53.4 to 100%, and at 3200 ppm from 30.7 to 100%. The 3200 ppm concentration remained the most consistently effective, with the majority of isolates exceeding 79% inhibition. The 2800 ppm concentration showed promising results, with all five isolates exceeding 50% inhibition. Notably, three of five isolates did not achieve 100% inhibition with 2000 ppm of the clove oil product in this experiment, a finding likely attributable to natural isolate-level variability in sensitivity rather than any fundamental limitation of the product. These data collectively established 3200 ppm as the most effective *in vitro* concentration, with 2800 ppm meriting further investigation as a lower-concentration alternative.

3.3 Experiment 3 — In Vivo Efficacy on Lemon Fruit

The *in vivo* experiment confirmed and extended the *in vitro* findings. ANOVA revealed a significant isolate × treatment interaction, reflecting the contrasting sensitivity profiles of the two selected isolates. The untreated, inoculated control experienced 0% inhibition (both wounds infected), as expected.

Figure 3 presents the mean percentage inhibition values for both isolates across all treatments. Isolate GM 250 (high sensitivity) experienced 70.7% inhibition at 1600 ppm lavender oil, 91.5% at 3200 ppm, and 80.7% at 6400 ppm. Isolate GM 263 (reduced sensitivity) experienced 8.8% inhibition at 1600 ppm, 62.6% at 3200 ppm, and 38.3% at 6400 ppm. Both isolates responded strongly to the 2000 ppm clove oil product (GM 250: 100%; GM 263: 99.6%). Notably, isolate GM 263 exhibited only 50% inhibition at 5 ppm imidazole, while GM 250

exhibited 73.7%, confirming that both isolates—particularly GM 263—display reduced sensitivity to the standard fungicide, further underscoring the need for effective alternatives.

A critical and unexpected finding was that 3200 ppm lavender oil outperformed 6400 ppm for both isolates *in vivo*. This inverse dose-response at the highest concentration is hypothesised to be related to phytotoxic damage to the lemon rind observed at 3200 and 6400 ppm concentrations (Figure 4), but absent at lower concentrations and entirely absent with imidazole or clove oil treatments. The rind damage may have compromised fruit tissue integrity, rendering the fruit more susceptible to infection through damaged tissue and thus artificially reducing the apparent inhibition percentage at 6400 ppm. The phytotoxicity is attributed to the use of crude, unformulated lavender oil; commercially registered essential oil fungicides such as the clove oil product undergo extensive formulation to mitigate phytotoxic effects. An additional beneficial observation was that lavender oil at 3200 and 6400 ppm inhibited sporulation of the fungus—a critical property for preventing secondary spread of infection within commercial export packaging. Imidazole is known to possess dual growth- and sporulation-inhibition activity, and the demonstration of a similar dual action by lavender oil at high concentrations strengthens its candidacy as a potential alternative.

[Figure 3 — Mean % inhibition of isolates GM 250 and GM 263 across treatments (see original data graph)]

Figure 3. Mean percentage inhibition of *Penicillium digitatum* isolates GM 250 (high sensitivity) and GM 263 (reduced sensitivity) by lavender oil (1600, 3200, 6400 ppm), imidazole (5 ppm), and clove oil product (2000 ppm) in Experiment 3.



Figure 4. Phytotoxic damage of lemon fruit rind following treatment with 3200 ppm (left) and 6400 ppm (right) lavender oil; damage was absent on imidazole- and clove oil-treated fruit. (Photo: Dr J.M. van Niekerk)

4. CONCLUSIONS

This study systematically evaluated the antifungal potential of *Lavandula angustifolia* essential oil against *Penicillium digitatum*—the principal causal agent of postharvest green mould in citrus—under both *in vitro* and *in vivo* conditions. The following principal conclusions are drawn:

(1) Concentration-Dependent Antifungal Activity: Lavender oil consistently demonstrated a positive concentration-response relationship across all experiments. The highest *in vitro* efficacy was observed at 3200 ppm, with isolate-specific inhibition values of up to 100% in the most sensitive isolates. This concentration-response pattern was reproducible across multiple experimental replications.

(2) Optimal Effective Concentration: The concentration of 3200 ppm was identified as the most reliably effective in both *in vitro* and *in vivo* settings. The 2800 ppm concentration also demonstrated considerable promise and warrants inclusion in future studies. *In vivo* results at 3200 ppm included dual-action inhibition of

both mycelial growth and sporulation—a property critical for preventing intra-carton disease spread in commercial citrus export contexts.

(3) Commercial Viability and Current Limitations: Although lavender oil exhibits meaningful antifungal activity against *P. digitatum*, its current form is not yet suitable for direct commercial application. Identified limitations include: the high concentration requirement (3200 ppm) relative to imidazole (5 ppm) and the clove oil product (2000 ppm); phytotoxic rind damage at effective concentrations, attributable to unformulated crude oil; differential isolate sensitivity necessitating broader population screening; and the high market cost of pure lavender oil. Professional formulation—analogueous to the development of the registered clove oil product—would likely mitigate phytotoxicity and potentially enhance efficacy at lower concentrations.

Future research should prioritise the formulation of lavender oil into a stable, commercially viable fungicidal preparation; evaluation of the 2800 ppm concentration in both in vitro and in vivo settings; investigation of the effect of fungal isolate age on treatment sensitivity; assessment of lavender oil efficacy against imidazole-resistant *P. digitatum* populations under commercial packhouse conditions; and a comprehensive cost-benefit analysis relative to existing registered fungicides and essential oil-based alternatives.

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