

Curative Treatment of Strawberry Gray Mold Caused by *Botrytis cinerea* Using Lavender Essential Oil

Miroslava Kačániová *, Minhang Qiao, Oleg Paulen

Slovak University of Agriculture in Nitra, Faculty of Horticulture and Landscape Engineering,
Institute of Horticulture, Slovakia

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Lavender essential oil exhibits strong *in vitro* antimicrobial activity against *Botrytis cinerea*; however, its efficacy under field conditions remains inconsistent. This study evaluated the effectiveness of lavender essential oil (*Lavandula angustifolia* × *latifolia* 'Vaucluse') against gray mold in strawberry (*Fragaria* × *ananassa* cv. 'Joly') under natural field conditions. *In vitro* assays confirmed pronounced antifungal activity, with a disc diffusion inhibition zone of 10.33 mm and minimum inhibitory concentrations of MIC₅₀ = 0.29 mg mL⁻¹ and MIC₉₀ = 0.38 mg mL⁻¹. Based on these results, field trials were conducted using a 10% (v/v) essential oil formulation applied either alone or as a curative treatment 24 h after inoculation with *B. cinerea* strain F-314. Despite strong *in vitro* efficacy, field application of lavender essential oil alone resulted in the highest gray mold incidence (53.9%), representing a 79% increase compared with untreated controls (30.1%) and a 108% increase relative to pathogen inoculation alone (25.9%). In contrast, curative application 24 h after pathogen inoculation produced the lowest disease incidence (13.7%), corresponding to a 55% reduction compared with untreated controls and a 47% reduction compared with pathogen-only treatment. The fourfold difference in disease incidence between essential oil application alone and curative treatment demonstrates that lavender essential oil can either exacerbate or suppress gray mold depending on application context. The increased disease incidence following essential oil application alone is likely attributable to phytotoxic stress and disruption of protective epiphytic microbiota, effects that were amplified by exceptionally high precipitation during the experimental period. Conversely, the efficacy of curative application may result from direct antimicrobial activity against established infections, reduced duration of phytotoxic exposure, and possible induction of host defense responses. These findings demonstrate that *in vitro* antimicrobial activity alone is insufficient to predict field performance and challenge the assumption that botanical antimicrobials effective in laboratory assays will necessarily confer disease protection when applied to plants. In strawberry gray mold management, lavender essential oil shows potential as a curative treatment but should not be applied in the absence of pathogen challenge, as this may increase disease susceptibility.

Keywords: lavender essential oil, *Botrytis cinerea*, strawberry, gray mold, *in vitro* antifungal activity, field efficacy, curative treatment, plant disease management

1 Introduction

Strawberry (*Fragaria* × *ananassa* Duch.) is one of the most economically important soft fruit crops worldwide, valued for its flavor, nutritional properties, and versatility. However, strawberry production is severely constrained by fungal diseases, among which gray mold caused by *Botrytis cinerea* Pers.: Fr. represents the most economically damaging pre- and post-harvest pathogen (Williamson et al., 2007). *Botrytis cinerea* is a necrotrophic fungal pathogen with an exceptionally broad host range

exceeding 200 plant species and is capable of infecting flowers, fruits, and vegetative tissues under favorable environmental conditions (Dean et al., 2012).

The pathogen thrives under conditions of high relative humidity, typically above 90%, and moderate temperatures ranging from 15 to 25 °C, which frequently coincide with strawberry flowering and fruit ripening periods. Infection by *B. cinerea* results in characteristic soft brown lesions that rapidly expand and become covered with gray sporulating mycelium, rendering

*Corresponding Author: Miroslava Kačániová, Slovak University of Agriculture in Nitra, Faculty of Horticulture and Landscape Engineering, Institute of Horticulture, Trieda Andreja Hlinku 2, 949 76 Nitra, Slovakia; e-mail: miroslava.kacaniova@gmail.com

fruits unmarketable and facilitating secondary infections during storage and transport (Romanazzi et al., 2016). Under favorable disease conditions, economic losses in strawberry production systems may exceed 20–50% of marketable yield, even when intensive fungicide application programs are implemented (Bursać Kovačević et al., 2016).

Management of *B. cinerea* has traditionally relied on repeated applications of synthetic fungicides, particularly compounds targeting sterol biosynthesis, such as demethylation inhibitors, and mitochondrial respiration, including succinate dehydrogenase inhibitors and quinone outside inhibitors. However, this strategy faces increasing limitations. *B. cinerea* exhibits remarkable genetic plasticity and has developed resistance to nearly all major fungicide classes, resulting in the widespread occurrence of multi-drug resistant strains with resistance to benzimidazoles, dicarboximides, anilinopyrimidines, and QoIs in strawberry-producing regions worldwide (Fernández-Ortuño et al., 2006; Weber & Hahn, 2011).

In addition, regulatory pressure has intensified due to concerns related to environmental contamination and human health risks, leading to the withdrawal or restriction of many effective fungicides. The European Union's "Farm to Fork" strategy, which aims to reduce pesticide use by 50% by 2030, further emphasizes the need for alternative disease management approaches (EC, 2020). At the same time, increasing consumer demand for organic and residue-free produce has created strong market incentives for production systems that minimize synthetic pesticide inputs (Yue et al., 2009). Synthetic fungicide use also generates significant environmental externalities, including negative impacts on non-target organisms, contamination of soil and water resources, and disruption of beneficial microbiome communities essential for plant health and ecosystem services (Altieri et al., 2024).

These converging pressures have intensified research efforts focused on sustainable and environmentally compatible alternatives for gray mold management in strawberry production. Among these alternatives, plant essential oils have emerged as promising biocontrol agents due to their broad-spectrum antimicrobial activity, rapid environmental degradation that minimizes residue concerns, and high consumer acceptance as natural products (Prakash et al., 2015; Regnault-Roger et al., 2012). Essential oils are complex mixtures of volatile secondary metabolites, predominantly mono- and sesquiterpenes, produced by aromatic plants as defense compounds against herbivores and pathogens.

Lavender essential oils derived from species of the genus *Lavandula* (Lamiaceae) have received particular attention because of their antimicrobial efficacy, low

dermal irritation, pleasant organoleptic properties, and established safety profiles in food and cosmetic applications. These oils are characterized by high concentrations of linalool, linalyl acetate, and lavandulyl acetate, with camphor and 1,8-cineole present as minor components. The major constituents exhibit multifaceted antimicrobial mechanisms, including disruption of cell membranes, inhibition of ATP synthesis, alteration of ion gradients, and interference with fungal morphogenesis (Nazzaro et al., 2013; Swamy et al., 2016).

The *in vitro* antifungal activity of essential oils against *B. cinerea* has been extensively documented. Numerous studies have reported strong inhibition of mycelial growth, spore germination, and sporulation using disc diffusion assays, vapor phase assays, and minimum inhibitory concentration determinations (Desam et al., 2019; Grušová et al., 2020). Minimum inhibitory concentrations of lavender essential oils against *B. cinerea* typically range from 0.1 to 2.0 $\mu\text{L mL}^{-1}$, depending on oil composition, fungal strain, and experimental methodology.

Despite these promising laboratory results, the translation of *in vitro* efficacy into reliable field-level disease control has often been inconsistent. Essential oil components are highly volatile and rapidly degrade under field conditions, resulting in limited residual activity (Isman & Machial, 2006). Their efficacy is strongly influenced by environmental factors such as temperature, humidity, ultraviolet radiation, and rainfall, which can significantly affect stability and bioavailability (Pavela, 2015). Furthermore, concentrations effective *in vitro* may cause phytotoxic effects when applied to living plant tissues, particularly under stressful environmental conditions (Verdeguer et al., 2020). Field applications also occur within complex plant – pathogen – microbiome systems that are absent from simplified laboratory assays (Stockwell et al., 2011), while limitations in formulation and application technology often hinder uniform coverage of hydrophobic essential oils on waxy fruit surfaces (Hyldgaard et al., 2012).

An emerging concept in botanical disease control is the critical role of application timing relative to pathogen infection dynamics. Most field studies have focused on prophylactic applications based on the assumption that *in vitro* antimicrobial activity would translate directly into preventive efficacy. However, such approaches may disrupt beneficial epiphytic microbiota, induce plant stress responses, or fail to coincide with vulnerable stages of pathogen development (Droby & Wisniewski, 2018). In contrast, curative application strategies applied after pathogen inoculation or symptom onset remain poorly explored, despite their potential advantages.

The present study was designed to address the gap between *in vitro* antimicrobial assessments and field

performance of essential oil-based control of gray mold in strawberries. The objectives were to evaluate the *in vitro* activity of lavender essential oil (*Lavandula angustifolia* × *latifolia* 'Vaucluse') against *B. cinerea*, to compare prophylactic and curative application strategies under field conditions, and to assess treatment performance under realistic environmental variability. We hypothesized that essential oil efficacy is strongly dependent on application timing and that curative applications may outperform prophylactic treatments, challenging conventional assumptions derived from laboratory data.

2 Material and Methods

2.1 Essential Oil Characterization

Lavender essential oil (*Lavandula angustifolia* × *latifolia* 'Vaucluse', CAS 8000-28-0) was obtained by steam distillation of fresh flowers and stems harvested from plants cultivated in the Karlovo region on the slopes of the Balkan Mountains, Bulgaria, and was purchased from Hanus s.r.o. (Nitra, Slovakia). The essential oil exhibited a soft lavender-bergamot aromatic profile dominated by linalyl acetate. Compositional analysis confirmed a chemical profile typical of lavender essential oil, consisting predominantly of linalyl acetate (20–40%) and linalool (18–40%), with lower proportions of lavandulyl acetate (1.5–6%), camphor (< 0.5%), and 1,8-cineole (< 1.5%). Prior to experimental use, the oil was stored at 4 °C in amber glass bottles under a nitrogen atmosphere to minimize oxidative degradation.

2.2 Fungal Strain and Culture Conditions

The fungal pathogen *Botrytis cinerea* Pers.: Fr. strain CCM F-314 (alternative designation F-50070) was obtained from the Czech Collection of Microorganisms (CCM, Brno, Czech Republic). The strain was originally isolated from cucumber (*Cucumis sativus*) in the Czech Republic and is characterized by α -amylase production capability. Stock cultures were maintained on malt extract agar and hay infusion agar (Oxoid, Basingstoke, UK) at 25 °C and subcultured monthly to preserve viability and pathogenicity.

For inoculation, spore suspensions were prepared from 7–10-day-old cultures by flooding plates with sterile distilled water containing 0.01% Tween 20, gently dislodging conidia with a sterile glass rod, and filtering the suspension through sterile cheesecloth to remove mycelial fragments. Spore concentration was adjusted to a 0.5 McFarland standard (approximately 1.5×10^8 CFU mL⁻¹) using a densitometer.

2.3 In vitro Antimicrobial Assays

The antifungal activity of lavender essential oil was evaluated using disc diffusion and minimum inhibitory concentration assays. For the disc diffusion assay, potato dextrose agar plates were inoculated with 0.1 mL of *B. cinerea* spore suspension adjusted to 1.5×10^8 spores mL⁻¹ and spread uniformly over the agar surface. Sterile filter paper discs (6 mm diameter, Whatman No. 1) were impregnated with 10 μ L of essential oil and placed on the inoculated agar. Plates were incubated at 25 °C for 24 h, after which inhibition zones were measured in millimeters from the disc edge to the advancing mycelial growth front.

Minimum inhibitory concentration was determined by incorporating the essential oil into molten PDA medium at final concentrations of 0.05, 0.1, 0.25, 0.5, 1.0, 2.0, and 5.0 μ L mL⁻¹. The oil was first solubilized in sterile Tween 20 solution (0.1% v/v) to ensure homogeneous dispersion. After solidification, agar plates were point-inoculated with 5 mm mycelial plugs excised from the actively growing margins of *B. cinerea* cultures and incubated at 25 °C. Mycelial growth was monitored daily for seven days, and the minimum inhibitory concentration was defined as the lowest essential oil concentration completely inhibiting visible fungal growth after the incubation period.

2.4 Field Site and Climatic Conditions

Field experiments were conducted at the Botanical Garden of the Slovak University of Agriculture in Nitra (BZ SPU), Slovakia, situated at an altitude of 144 m above sea level (48° 19' 15" N, 18° 06' 30" E). The site lies between the Zobor massif and the hills Kalvária and Šibeničný vrch and represents one of the warmest and driest agricultural

Table 1 Monthly temperature deviations from climatic normal (1991–2020) at Nitra, 2024

Month	Mean temperature (°C)	Normal 1991–2020 (°C)	Δt (°C)	Classification
March	0.9	5.5	-4.6	cold
April	11.7	11.4	+0.3	normal
May	17.6	16.0	+1.6	warm
June	20.8	19.6	+1.1	warm
July	23.8	21.7	+2.1	very warm

Source: Meteorological Station FHLE-ILE

Table 2 Monthly precipitation relative to long-term average (1951–2000) in Nitra, 2024

Month	Precipitation (mm)	Normal 1951–2000 (mm)	% of normal	Classification
March	29	33	87	normal
April	56	36	154	very wet
May	62	59	104	normal
June	181	59	306	extremely wet
July	17	65	26	very dry

Source: Meteorological Station FHLE-ILEt

regions of Slovakia, where irrigation is frequently required during summer due to precipitation deficits.

The soil at the experimental site is classified as gleyed fluvizem developed from alluvial deposits of the Nitra River, containing both calcareous and non-calcareous components. The soil exhibits a high clay content, resulting in a sticky structure when wet and a hard, compact consistency when dry.

Meteorological data were recorded by the Department of Biometeorology and Hydrology at the on-site weather station of BG SUA. During the experimental period from March to July 2024, climatic conditions deviated markedly from the long-term normal (1991–2020). March was exceptionally cold, with a mean temperature 4.6 °C below normal, while May, June, and July were warmer than average. Precipitation patterns were highly variable, with April and June being very wet and extremely wet, respectively, whereas July was characterized by severe drought. The exceptionally high precipitation recorded in June coincided with the fruit development and ripening period, creating prolonged conditions of high humidity and leaf wetness favorable for *B. cinerea* infection and disease development.

2.5 Plant Material and Experimental Design

Strawberry plants (*Fragaria* × *ananassa* cv. 'Joly') were established in March 2021 in the demonstration garden area of BG SUA at a spacing

of 0.5 × 0.3 m and maintained as a matted-row system. The cultivar 'Joly' is characterized by high yield potential, attractive fruit quality with excellent flavor, and moderate resistance to fungal diseases, making it suitable for evaluating plant protection strategies under

realistic production conditions. From 2021 to 2023, plants received soil applications of biostimulants, while no biostimulant or fungicide treatments were applied during the 2024 season to allow natural pathogen pressure.



Figure 1 Experimental groups of strawberries after treatment
Source: Kačániová, 2024

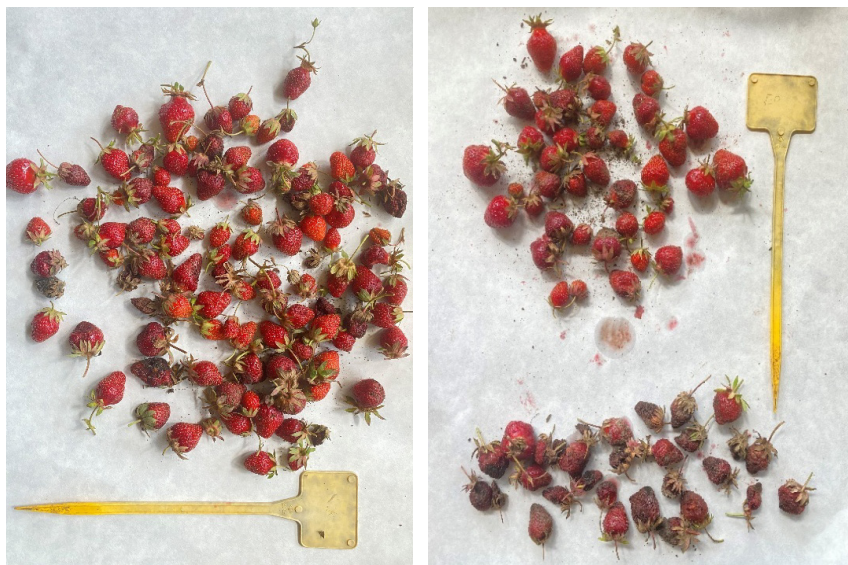


Figure 2 Evaluation of the experiment
Source: Kačániová, 2024

The experiment was arranged in a completely randomized design with three replications, each consisting of 20 plants per treatment. Four treatment variants were established to compare prophylactic and curative essential oil application strategies: untreated control plants exposed to natural pathogen pressure; plants inoculated with *B. cinerea* without essential oil treatment; plants treated prophylactically with lavender essential oil 24 h before pathogen inoculation; and plants inoculated with *B. cinerea* followed by curative essential oil application 24 h later.

2.6 Treatment Preparation and Application

A working solution of lavender essential oil at a concentration of 10% (v/v) was prepared fresh on the day of application by emulsifying the oil in distilled water containing 0.1% Tween 20 with vigorous agitation to improve dispersion and coverage on waxy plant surfaces.

During the final flowering stage on 9 May 2024, all experimental plants were surface-disinfected with a 3% sodium hypochlorite solution to eliminate existing epiphytic microorganisms and standardize initial microbial conditions.

Applications were performed using a hand-operated sprayer equipped with an atomizing nozzle held approximately 35 cm above the plant canopy, delivering 15 mL of solution per plant. The sprayer produced a fine mist with an estimated droplet size of 150–200 μm , and each application lasted 5–10 s with continuous sweeping motion to ensure uniform coverage of flowers, developing fruits, and foliage.

Prophylactic essential oil treatment was applied on the same day, 24 h prior to pathogen inoculation, using identical spraying parameters. Artificial inoculation with *B. cinerea* spore suspension adjusted to 0.5 McFarland standard was performed on 10 May 2024 for the designated treatment groups. Control and essential-oil-only plants were not artificially inoculated and were exposed solely to natural environmental inoculum present at the field site. Curative essential oil treatment was applied to the appropriate treatment group on 11 May 2024, following a 24 h incubation period allowing initial pathogen infection processes.

2.7 Disease Assessment

Fruits were harvested 18 days after treatment application, on 29 May 2024, when fruits reached commercial ripeness. Harvested fruits from each treatment were collected separately and visually assessed individually for typical gray mold symptoms, including soft brown lesions, tissue maceration, discoloration, and the presence of gray sporulating mycelium. Fruits exhibiting any disease symptoms were classified as diseased irrespective of lesion size.

Disease incidence was calculated as the percentage of diseased fruits relative to the total number of harvested fruits per treatment.

3 Results and Discussion

3.1 *In vitro* Antimicrobial Activity of Lavender Essential Oil

Lavender essential oil of the cultivar 'Vaucluse' exhibited clear antifungal activity against *Botrytis cinerea* strain F-314 under *in vitro* conditions. Disc diffusion assays produced distinct inhibition zones with a mean diameter of 10.33 mm, indicating effective suppression of mycelial growth. Minimum inhibitory concentration assays further confirmed the antifungal potency of the oil, with MIC_{50} and MIC_{90} values of 0.29 $\text{mg}\cdot\text{mL}^{-1}$ and 0.38 $\text{mg}\cdot\text{mL}^{-1}$, respectively. These values are in close agreement with previously reported inhibitory concentrations of lavender essential oils against *B. cinerea*, as documented by Lis-Balchin & Roth (2000) and Grulová et al. (2020), confirming that the tested oil possessed typical and biologically relevant antimicrobial activity. Based on these laboratory results, lavender essential oil was selected for field evaluation at a concentration of 10% (v/v), corresponding to approximately 100 $\text{mg}\cdot\text{mL}^{-1}$. This concentration represents an approximately 260-

fold excess relative to the MIC₉₀ value determined *in vitro* and was chosen to compensate for the expected reduction in bioavailability under field conditions caused by volatilization, photodegradation, and environmental dilution.

3.2 Field Efficacy of Lavender Essential Oil under Natural Disease Pressure

Field trials conducted under natural environmental conditions in May 2024 revealed significant differences between treatment groups. Disease incidence evaluated 18 days post-treatment showed marked variations among groups. Untreated control plants exhibited gray mold incidence of 30.1%, indicating substantial natural disease pressure during the evaluation period. Artificial inoculation with *B. cinerea* alone resulted in slightly lower disease incidence of 25.9%, a difference remaining within biological variability under field conditions.

In contrast, plants treated only with lavender essential oil without artificial *B. cinerea* inoculation exhibited the highest gray mold incidence (53.9%). This represents a 79% increase compared to the untreated control (30.1%) and a 108% increase compared to artificial *B. cinerea* inoculation without essential oil (25.9%). This result sharply contradicts expectations based on the strong *in vitro* antifungal activity of the oil and indicates that essential oil application alone not only failed to protect strawberry fruits but significantly exacerbated disease development under the prevailing environmental conditions.

3.3 Adverse Effects of Prophylactic Essential Oil Application

The increased disease incidence observed following prophylactic essential oil treatment suggests that the application induced conditions favorable for *B. cinerea* infection rather than suppressing it. One plausible explanation is the induction of phytotoxic stress under conditions of prolonged surface wetness and high humidity. The experimental period coincided with exceptionally high precipitation, with June 2024 recording 181 mm of rainfall, corresponding to 306% of the long-term normal. Such conditions result in extended leaf and fruit wetness, which may amplify tissue sensitivity to chemical stressors.

At the applied concentration of 10%, lavender essential oil substantially exceeded the minimum antimicrobial requirement determined *in vitro*. Monoterpenes such as linalool and linalyl acetate are known to disrupt cellular membranes and alter permeability not only in fungal cells but also in plant tissues at elevated concentrations (Nazzaro et al., 2013; Tworowski, 2002). Under prolonged humid conditions, this disruption may compromise fruit cuticle integrity, facilitate tissue maceration, and increase susceptibility to necrotrophic pathogens such as *B. cinerea*. Consistent with this interpretation, Dèné et al. (2023) reported visible phytotoxic effects on strawberry plants at essential oil concentrations exceeding 2% (v/v).

Second, non-exclusive mechanism involves disruption of the protective epiphytic microbiome. The experimental protocol included surface disinfection of all plants with sodium hypochlorite prior to treatment application, eliminating pre-existing microbial communities. While control plants were subsequently recolonized by naturally occurring epiphytic microorganisms, the broad-spectrum antimicrobial activity of lavender essential oil may have inhibited the re-establishment of beneficial microbial populations on fruit surfaces in the prophylactic treatment. The strawberry phyllosphere microbiome plays a crucial role in suppressing gray mold through competitive exclusion and production of antifungal metabolites (Abdelfattah et al., 2016; Wei et al., 2016). Elimination or suppression of these communities likely reduced biological resistance to *B. cinerea*, contributing to the elevated disease incidence observed.

3.4 Superior Efficacy of Curative Essential Oil Application

In contrast to prophylactic treatment, curative application of lavender essential oil 24 h after pathogen inoculation resulted in the lowest disease incidence among all treatments, with only 13.7% of fruits exhibiting gray mold symptoms. This corresponds to a 55% reduction relative to untreated controls, a 47% reduction compared with pathogen inoculation alone, and a 75% reduction relative to prophylactic essential oil treatment. These results clearly demonstrate that the same essential oil concentration can exert either deleterious or highly protective effects depending solely

Table 3 Gray mold incidence on strawberry fruits 18 days post-treatment (May 29, 2024)

Treatment	Description	Diseased fruits	Total fruits	Disease incidence (%)
Control	Untreated	68	226	30.1
H	<i>B. cinerea</i> F-314 inoculation	50	193	25.9
EO	10% LEO prophylactic	117	217	53.9
H + EO	<i>B. cinerea</i> + curative LEO	25	183	13.7

on application timing. The efficacy of curative treatment may be attributed primarily to direct antimicrobial activity against *B. cinerea* during early infection stages. Application 24 h after inoculation targets the pathogen during active germination and hyphal expansion, stages that are particularly sensitive to membrane-disrupting compounds such as linalool and linalyl acetate (Nazzaro et al., 2013). At this stage, fungal metabolism is highly active, potentially increasing vulnerability to essential oil constituents. Additionally, curative application likely reduced the duration of plant tissue exposure to high essential oil concentrations compared with prophylactic treatment, thereby minimizing phytotoxic stress while retaining antimicrobial efficacy. A further contributing factor may be the activation of induced resistance mechanisms. *Botrytis cinerea* is known to trigger defense signaling pathways in strawberry plants (Haile et al., 2020), and monoterpenes present in essential oils have been reported to function as defense elicitors (Vats, 2018). The sequential exposure to pathogen challenge followed by essential oil treatment may therefore have resulted in synergistic activation of host defenses, although confirmation of this mechanism requires molecular analysis.

3.5 Influence of Environmental Conditions

Weather conditions during the experimental period strongly favored gray mold development. Elevated precipitation combined with moderately increased temperatures created prolonged periods of high relative humidity and leaf wetness, which are optimal for *B. cinerea* infection and sporulation (Williamson et al., 2007). The substantial disease incidence observed in untreated control plants confirms the presence of strong natural infection pressure. Under such conditions, any treatment-induced tissue stress or disruption of protective barriers is likely to be rapidly exploited by the pathogen, amplifying differences between treatment strategies.

3.6 Comparison with Previous Studies and Implications for Disease Management

The pronounced timing-dependent effects observed in this study have rarely been documented with such magnitude in essential oil field trials. Most previous studies have relied on prophylactic application strategies based primarily on *in vitro* antimicrobial efficacy (Prakash et al., 2015). While Areco et al., (2024) demonstrated effective control of *B. cinerea* using essential oil vapors in closed systems, they reported reduced efficacy under open-field conditions. Similarly (Desam et al., 2019) observed variable disease suppression using repeated low-concentration applications.

The present results demonstrate that a single curative application can achieve greater disease reduction than prophylactic treatment, highlighting application timing as a critical determinant of field efficacy. These findings suggest that essential oil-based treatments should be aligned with infection dynamics rather than applied preventively and that concentration optimization and formulation development will be essential for practical deployment.

3.7 Study Limitations and Future Perspectives

This study evaluated a single essential oil concentration and two application timings under specific environmental conditions characterized by exceptionally high precipitation. While these conditions provided a robust test of treatment performance under severe disease pressure, validation across multiple seasons, locations, and concentration ranges is required. The proposed mechanisms involving microbiome disruption and induced resistance remain hypothetical and should be confirmed through targeted microbiome profiling and defense gene expression analyses in future studies.

4 Conclusions

This study reveals a striking paradox in the application of lavender essential oil for the management of strawberry gray mold. Despite exhibiting strong *in vitro* antifungal activity against *Botrytis cinerea* ($MIC_{90} = 0.38 \text{ mg mL}^{-1}$), the essential oil produced diametrically opposite outcomes under field conditions depending solely on application timing. Prophylactic application of lavender essential oil at a concentration of 10% (v/v) resulted in a 79% increase in gray mold incidence compared with untreated controls, whereas curative application at the same concentration reduced disease incidence by 55% relative to controls. These findings clearly demonstrate that *in vitro* antimicrobial efficacy alone is insufficient to predict field-level disease control performance. Instead, the timing of essential oil application in relation to pathogen infection dynamics is a critical determinant of treatment success or failure. In the context of strawberry gray mold management, curative application of lavender essential oil following pathogen challenge provided effective disease suppression, while prophylactic application increased host susceptibility under the prevailing environmental conditions. The results underscore the need for a paradigm shift in the evaluation and deployment of botanical antimicrobials in crop protection. Future research should prioritize optimization of essential oil concentrations to identify minimum effective doses, detailed temporal studies to define optimal curative application windows, and mechanistic investigations addressing phytotoxic

effects and interactions with the plant-associated microbiome. Furthermore, validation of these findings across multiple growing seasons, locations, and climatic conditions will be essential to assess the robustness and practical applicability of essential oil-based disease management strategies.

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References

- Abdelfattah, A., Wisniewski, M., Droby, S., & Schena, L. (2016). Spatial and compositional variation in the fungal communities of organic and conventionally grown apple fruit at the consumer point-of-purchase. *Horticulture Research*, 3(1), 16047. <https://doi.org/10.1038/hortres.2016.47>
- Altieri, V., Rossi, V., & Fedele, G. (2024). Integration of mathematical modeling and target-based application of biocontrol agents for the control of *Botrytis cinerea* in vineyards. *Pest Management Science*, 80(9), 4352–4360. <https://doi.org/10.1002/ps.8140>
- Areco, V. A., Achimón, F., Almirón, C., Nally, M. C., Zunino, M. P., & Yaryura, P. (2024). Antifungal activity of essential oils rich in ketones against *Botrytis cinerea*: New strategy for biocontrol. *Biocatalysis and Agricultural Biotechnology*, 59, 103233. <https://doi.org/10.1016/j.bcab.2024.103233>
- Bursač Kovačević, D., Putnik, P., Dragović-Uzelac, V., Pedisić, S., Režek Jambrak, A., & Herceg, Z. (2016). Effects of cold atmospheric gas phase plasma on anthocyanins and color in pomegranate juice. *Food Chemistry*, 190, 317–323. <https://doi.org/10.1016/j.foodchem.2015.05.099>
- Dean, R., Van Kan, J. A. L., Pretorius, Z. A., Hammond-Kosack, K. E., Di Pietro, A., Spanu, P. D., Rudd, J. J., Dickman, M., Kahmann, R., Ellis, J., & Foster, G. D. (2012). The Top 10 fungal pathogens in molecular plant pathology: Top 10 fungal pathogens. *Molecular Plant Pathology*. <https://doi.org/10.1111/j.1364-3703.2012.2011.00783.x>
- Dènè, L., Laužikė, K., Rasiukevičiūtė, N., Chrapačienė, S., Brazaitytė, A., Viršilė, A., Vaštakaitė-Kairienė, V., Miliauskienė, J., Sutulienė, R., Samuolienė, G., & Valiūškaitė, A. (2023). Defense response of strawberry plants against *Botrytis cinerea* influenced by coriander extract and essential oil. *Frontiers in Plant Science*, 13, 1098048. <https://doi.org/10.3389/fpls.2022.1098048>
- Desam, N. R., Al-Rajab, A. J., Sharma, M., Mylabathula, M. M., Gowkanapalli, R. R., & Albratty, M. (2019). Chemical constituents, *in vitro* antibacterial and antifungal activity of *Mentha × Piperita* L. (peppermint) essential oils. *Journal of King Saud University – Science*, 31(4), 528–533. <https://doi.org/10.1016/j.jksus.2017.07.013>
- Droby, S., & Wisniewski, M. (2018). The fruit microbiome: A new frontier for postharvest biocontrol and postharvest biology. *Postharvest Biology and Technology*, 140, 107–112. <https://doi.org/10.1016/j.postharvbio.2018.03.004>
- EC, E. C. (2020). Farm to fork strategy for a fair, healthy and environmentally-friendly food system. *Communication from the EU Commission*, COM, 381.
- Fernández-Ortuño, D., Pérez-García, A., López-Ruiz, F., Romero, D., De Vicente, A., & Torés, J. (2006). Occurrence and distribution of resistance to QoI fungicides in populations of *Podosphaera fusca* in south central Spain. *European Journal of Plant Pathology*, 115(2), 215–222.
- Gruľová, D., Caputo, L., Elshafie, H. S., Baranová, B., De Martino, L., Sedlák, V., Gogaľová, Z., Poráčová, J., Camele, I., & De Feo, V. (2020). Thymol Chemotype *Origanum vulgare* L. Essential Oil as a Potential Selective Bio-Based Herbicide on Monocot Plant Species. *Molecules*, 25(3), 595. <https://doi.org/10.3390/molecules25030595>
- Haile, Z. M., Malacarne, G., Pilati, S., Sonego, P., Moretto, M., Masuero, D., Vrhovsek, U., Engelen, K., Baraldi, E., & Moser, C. (2020). Dual Transcriptome and Metabolic Analysis of *Vitis vinifera* cv. Pinot Noir Berry and *Botrytis cinerea* During Quiescence and Egressed Infection. *Frontiers in Plant Science*, 10, 1704. <https://doi.org/10.3389/fpls.2019.01704>
- Hyldgaard, M., Mygind, T., & Meyer, R. L. (2012). Essential Oils in Food Preservation: Mode of Action, Synergies, and Interactions with Food Matrix Components. *Frontiers in Microbiology*, 3. <https://doi.org/10.3389/fmicb.2012.00012>
- Isman, M. B., & Machial, C. M. (2006). Chapter 2 Pesticides based on plant essential oils: From traditional practice to commercialization. In *Advances in Phytomedicine* (vol. 3, pp. 29–44). [https://doi.org/10.1016/S1572-557X\(06\)03002-9](https://doi.org/10.1016/S1572-557X(06)03002-9)
- Lis-Balchin, M., & Roth, G. (2000). Composition of the essential oils of *Pelargonium odoratissimum*, *P. exstipulatum*, and *P. fragrans* (Geraniaceae) and their bioactivity. *Flavour and Fragrance Journal*, 15(6), 391–394. [https://doi.org/10.1002/1099-1026\(200011/12\)15:6%253C391::AID-FFJ929%253E3.0.CO;2-W](https://doi.org/10.1002/1099-1026(200011/12)15:6%253C391::AID-FFJ929%253E3.0.CO;2-W)
- Nazzaro, F., Fratianni, F., De Martino, L., Coppola, R., & De Feo, V. (2013). Effect of Essential Oils on Pathogenic Bacteria. *Pharmaceuticals*, 6(12), 1451–1474. <https://doi.org/10.3390/ph6121451>
- Pavela, R. (2015). Essential oils for the development of eco-friendly mosquito larvicides: A review. *Industrial Crops and Products*, 76, 174–187. <https://doi.org/10.1016/j.indcrop.2015.06.050>
- Prakash, B., Kedia, A., Mishra, P. K., & Dubey, N. K. (2015). Plant essential oils as food preservatives to control moulds, mycotoxin contamination and oxidative deterioration of agri-food commodities – Potentials and challenges. *Food Control*, 47, 381–391. <https://doi.org/10.1016/j.foodcont.2014.07.023>
- Regnault-Roger, C., Vincent, C., & Arnason, J. T. (2012). Essential Oils in Insect Control: Low-Risk Products in a High-Stakes World. *Annual Review of Entomology*, 57(1), 405–424. <https://doi.org/10.1146/annurev-ento-120710-100554>
- Romanazzi, G., Sanzani, S. M., Bi, Y., Tian, S., Gutiérrez Martínez, P., & Alkan, N. (2016). Induced resistance to control postharvest decay of fruit and vegetables. *Postharvest Biology and Technology*, 122, 82–94. <https://doi.org/10.1016/j.postharvbio.2016.08.003>
- Stockwell, V. O., Johnson, K. B., Sugar, D., & Loper, J. E. (2011). Mechanistically Compatible Mixtures of Bacterial

Antagonists Improve Biological Control of Fire Blight of Pear. *Phytopathology*, 101(1), 113–123.

<https://doi.org/10.1094/PHTO-03-10-0098>

Swamy, M. K., Akhtar, M. S., & Sinniah, U. R. (2016). Antimicrobial Properties of Plant Essential Oils against Human Pathogens and Their Mode of Action: An Updated Review. *Evidence-Based Complementary and Alternative Medicine*, 2016(1), 3012462. <https://doi.org/10.1155/2016/3012462>

Tworokski, T. (2002). Herbicide effects of essential oils. *Weed Science*, 50(4), 425–431. [https://doi.org/10.1614/0043-1745\(2002\)050%255B0425:HEOE0%255D2.0.CO;2](https://doi.org/10.1614/0043-1745(2002)050%255B0425:HEOE0%255D2.0.CO;2)

Vats, S. (Éd.). (2018). *Biotic and Abiotic Stress Tolerance in Plants*. <https://doi.org/10.1007/978-981-10-9029-5>

Verdeguer, M., Sánchez-Moreiras, A. M., & Araniti, F. (2020). Phytotoxic Effects and Mechanism of Action of Essential Oils and Terpenoids. *Plants*, 9(11), 1571.

<https://doi.org/10.3390/plants9111571>

Weber, R. W. S., & Hahn, M. (2011). A rapid and simple method for determining fungicide resistance in *Botrytis*. *Journal of Plant Diseases and Protection*, 118(1), 17–25.

<https://doi.org/10.1007/BF03356376>

Wei, F., Hu, X., & Xu, X. (2016). Dispersal of *Bacillus subtilis* and its effect on strawberry phyllosphere microbiota under open field and protection conditions. *Scientific Reports*, 6(1), 22611. <https://doi.org/10.1038/srep22611>

Williamson, B., Tudzynski, B., Tudzynski, P., & Van Kan, J. A. (2007). *Botrytis cinerea*: The cause of grey mould disease. *Molecular plant pathology*, 8(5), 561–580.

Yue, C., Alfnes, F., & Jensen, H. H. (2009). Discounting Spotted Apples: Investigating Consumers' Willingness to Accept Cosmetic Damage in an Organic Product. *Journal of Agricultural and Applied Economics*, 41(1), 29–46. <https://doi.org/10.1017/S1074070800002534>

