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Essential Oil-Based Nanoemulsions as Sustainable Control Method Against *Colletotrichum gloeosporioides* and *Neofusicoccum parvum* on Citrus †

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Abstract

Fungal diseases represent one of the major threats to citrus production, such as anthracnose caused by *Colletotrichum gloeosporioides* and Fungal Trunk Diseases (FTDs) associated with Botryosphaeriaceae, with *Neofusicoccum parvum* being the most prevalent species. In response to the need to reduce chemical fungicide use, this study evaluated the antifungal activity of essential oil-based nanoemulsions (N-EOs) as alternative management methods. Seven N-EOs (citronella, clove, fennel, garlic, laurel, lavender and peppermint) were first screened *in vitro* against multiple isolates of both pathogens through mycelial growth and conidial germination assays. Based on estimated EC_{50} and EC_{90} values, clove and garlic N-EOs exhibited the highest inhibitory activity, while lavender displayed intermediate but promising efficacy, particularly against *N. parvum*. These N-EOs were subsequently evaluated *in vivo* on lemon fruits inoculated with *C. gloeosporioides* and on detached lemon twigs inoculated with *N. parvum*. *In vivo* assays largely confirmed the *in vitro* trends, with clove and garlic significantly reducing lesion development. In contrast, lavender displayed limited efficacy under *in vivo* conditions. The phytotoxic effects at higher concentrations limited the range of applicable doses. Overall, the results suggest that N-EOs, particularly those based on clove and garlic, may offer potential as alternative tools for citrus disease management. However, host tissue interactions, formulation stability, volatility, and validation under field conditions remain critical aspects requiring further investigation.

Keywords: citrus diseases; essential oil nanoemulsion; antifungal activity; *Colletotrichum gloeosporioides*; *Neofusicoccum parvum*; integrated disease management

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1. Introduction

Citrus (*Citrus* L.) crops originated in Eurasia, specifically in southern China or the surrounding territories [1], and are nowadays distributed in various countries around the world. The global citrus trade has grown consistently over recent decades, as evidenced by the fact that they are now cultivated in over 140 countries, with Brazil, China, India, Iran,

Italy, Mexico, Spain and the United States being among the world's leading producers [2]. Furthermore, 52% of citrus fruit exports (7.2 million tonnes) originate from the Mediterranean region [3], highlighting the strategic importance of the citrus sector for the Italian economy and production systems. Around 55% of the national citrus-growing area (approximately 61,000 hectares) is in Sicily [4]. This emphasises the strategic importance of this sector for both the region and the whole country. However, it faces multiple challenges annually, with fungal diseases being one of the most significant due to their direct and indirect impacts [5]. Among these, *Colletotrichum* spp., causal agents of anthracnose, are one of the major fungal pathogens [6], affecting fruits, canopy and twigs, leading to premature fruit drop and canopy decline [7–9]. Specifically, in Sicily, the predominant fungal species causing anthracnose is *Colletotrichum gloeosporioides* [6,10,11]. Furthermore, in recent decades, there has been an increase in cases of Fungal Trunk Diseases (FTDs), which are caused by a variety of fungal species mainly belonging to the Botryosphaeriaceae family, which affect numerous crops [12–15], including citrus [16–19]. FTDs are characterized by symptoms such as branch, twig and trunk cankers, shoot dieback and gummosis [20], with *Neofusicoccum parvum* being the predominant and most aggressive species associated with FTDs [19,21–23]. Conventionally, the primary strategy employed in the management of fungal infections has been the application of chemical compounds. However, it has become increasingly evident that the improper use of pesticides can lead to severe environmental and human health hazards [24]. Therefore, in line with one of the key objectives of the Farm to Fork strategy adopted by the European Union, which aims to reduce the risk and use of chemical pesticides by 50% by 2030 [25], and with the growing interest in alternative control methods, the main objective of this work was to study the antifungal activity of various essential oil-based products. These compounds are well-known for their antifungal and antibacterial properties, primarily due to the variety of active ingredients and secondary metabolites they contain. Some of the most studied essential oils for controlling *Colletotrichum* spp. are clove [26–30] and citronella or related *Cymbopogon* essential oils, such as lemongrass [31–33]. Both exhibit strong antifungal activity, largely attributed to their major bioactive constituents, eugenol in clove [34] and citronellal, citronellol, and geraniol in citronella [35]. In particular, clove oil has shown marked activity against *C. gloeosporioides*, with MIC values of 80 μL L^{-1} in the vapour phase and 300 μL L^{-1} in direct contact assays [36]. Moreover, eugenol, the major constituent of clove oil, was reported to inhibit *N. parvum*, also recognized as the grapevine trunk disease pathogen, showing fungistatic activity at 1500 μg mL^{-1}, fungicidal activity at 2500 μg/mL and inhibition of conidial germination at 750 μg mL^{-1} [37]. Similarly, garlic and lavender have also been widely investigated for their antifungal properties [27,38,39], with lavender mainly characterized by the presence of linalool and other terpenoids [40] and garlic by a high content of organosulfur compounds [41]. Consistently, garlic essential oil showed an EC_{50} value of 10.10 μL L^{-1} for the reduction in mycelial growth in the contact phase [42]. Likewise, other essential oils have demonstrated promising antimicrobial potential, including those from fennel [27,43,44], laurel [27,38,45] and peppermint [46,47]. For instance, peppermint essential oil completely inhibited the mycelial growth of *Colletotrichum* isolates from mango at 5 μL/mL [48]. In contrast, previous studies specifically focused on *N. parvum* are still limited. Nevertheless, the available data indicate that some oils or their main constituents, such as eugenol in clove [37] and peppermint essential oil [49], can directly inhibit its growth, suggesting that essential oil-based products may also be promising tools for controlling pathogens associated with FTDs. Furthermore, it is evident that over the last decade, research on essential oils has increased markedly, particularly in *in vitro* assays and in active food packaging applications [50]. However, the practical use of these compounds remains limited by several intrinsic limitations, including high volatility,

susceptibility to degradation and poor solubility in water, which can reduce their stability and bioavailability [51]. Despite these limitations, essential oils offer several advantages as natural antimicrobial agents, such as broad-spectrum activity and a reduced risk of resistance development [52]. To overcome their physicochemical constraints, the present study focused on essential oil-based nanoemulsions (N-EOs), which could represent an effective strategy to improve their performance compared with pure oils. N-EOs could enhance water solubility, reduce droplet coalescence and phase separation, and increase the surface area of bioactive compounds, thereby improving their interaction with target microorganisms and allowing the use of lower concentrations [53].

Consequently, the present study aimed to evaluate seven different nanoemulsions, formulated with citronella, clove, fennel, garlic, laurel, lavender, and peppermint essential oils against the aforementioned fungal species.

Specifically, this research investigated the *in vitro* antifungal activity of the seven N-EOs through mycelial growth and conidial germination assays (i), as well as their *in vivo* efficacy on lemon fruits against *Colletotrichum gloeosporioides* (ii) and on detached lemon twigs against *Neofusicoccum parvum* (iii). This study provides new relevant insights into the bioprotection against two major citrus diseases, helping to identify sustainable management strategies for this high-impact Mediterranean crop.

2. Materials and Methods

2.1. Formulation and Physical Characterization of Nanoemulsion Based on Essential Oil (N-EOs)

All pure essential oils (EO) in this study were purchased by Esperis S.p.A. (Milan, Italy) (Table 1). Nanoemulsions were prepared in the laboratories of Entomologia Generale e Applicata, Department of AGRARIA, University of Reggio Calabria, Italy, using the high-pressure microfluidization (HPM) technique, following the methods described by Modafferi et al. [54]. The essential oil (EO) was mixed with Tween 80® (Polyoxyethylene (20) sorbitan monooleate, Sigma-Aldrich, Munich, Germany) at a 3:1 (*w*:*w*) ratio using a magnetic stirrer (6000 rpm, 5 min) to obtain a homogeneous organic phase. Double-distilled water was then added dropwise (1 mL/min) to the organic phase at a 4:1 (*w*:*w*) ratio to form a raw emulsion (15% EO, 5% Tween 80®, and 80% water, *w*:*w*:*w*), which was further mixed for 5 min at 7000 rpm. The pre-emulsion was subsequently homogenized five times at 30,000 psi using a high-pressure microfluidizer (LM20 Microfluidizer™ Processor, Westwood, MA, USA) with the chamber cooled in an ice bath (<10 °C). The resulting nanoemulsions were transferred to aluminium containers and stored at room temperature, protected from direct sunlight and heat sources, following the same procedure for each essential oil. Then, the droplet size (Z-average), polydispersity index (PDI) and surface charge (ζ-potential) were measured to investigate the physical properties—average diameter, size distribution uniformity, and electrostatic surface charge of the droplets. The nanoemulsions were diluted in double-distilled water (ratio 1:200 *v*:*v*), and the dynamic light scattering (DLS) equipment (Zetasizer Nano, Malvern®, Malvern, UK) was used.

Table 1. List of essential oils, their corresponding plant species, and batch identification numbers supplied by Esperis S.p.A. (Milan, Italy).

Essential Oils	Plant Species	Batch No.
Citronella	*Cymbopogon winterianus* Jowitt ex Bor	OL. ES. 46
Clove	*Syzygium aromaticum* (L.) Merr. & L.M. Perry	OL. ES. 57
Fennel	*Foeniculum vulgare* Mill.	OL. ES. 54
Garlic	*Allium sativum* L.	OL. ES. 4
Laurel	*Laurus nobilis* L.	OL. ES. 67
Lavender	*Lavandula angustifolia* Mill.	OL. ES. 307
Peppermint	*Mentha* × *piperita* L.	OL. ES. 94

2.2. Fungal Isolates

Two fungal species were considered to evaluate the antifungal activity of the N-EOs: *C. gloeosporioides* and *N. parvum*. Both species were originally isolated from symptomatic *Citrus* spp. tissues from two representative Mediterranean citrus growing areas, Italy and Spain. A comprehensive list of all isolates, including host plant, geographic origin, and reference, is provided in Table 2. For *N. parvum*, two fungal isolates recovered from symptomatic *Citrus* twigs in Italian orchards were selected [19]. These isolates belong to the fungal collection of the Plant Pathology laboratory, Department of Agriculture, Food and Environment, University of Catania (Unict). For *C. gloeosporioides*, two Italian and two Spanish isolates were included in the study, enabling assessment of potential differences in N-EO sensitivity related to geographic origin. One of the Spanish isolates was previously molecularly characterized [55], whereas the remaining Spanish and the two Italian ones were molecularly identified in the present study, and their sequences were deposited as described in the following section. All cultures were grown on potato dextrose agar (PDA, Oxoid, Basingstoke, UK) at 24 ± 2 °C with a 12 h photoperiod of fluorescent lighting and transferred every 7–10 days to maintain viability for experimental assays.

Table 2. Comprehensive list of fungal isolates used in this study, including host plant, geographic origin, and reference.

Fungal Species	Isolate	Host	Symptoms	Location	References
Colletotrichum gloeosporioides	Col-69	*Citrus sinensis*	Fruit lesions	Fuente Palmera, Córdoba, Spain	[55]
	PV-1457	*Citrus sinensis*	Fruit lesions	Palma del Río, Córdoba, Spain	Present study *
	LA2	*Citrus sinensis*	Fruit lesions	Sicily, Italy	Present study *
	LI3	*Citrus sinensis*	Fruit lesions	Sicily, Italy	Present study *
Neofusicoccum parvum	AGR-CT-27	*Citrus limon*	Internal wood necrosis	Sicily, Italy	[19]
	AGR-CT-84	*Citrus limon*	Internal wood necrosis	Sicily, Italy	[19]

* Isolate that was molecularly identified in the present work; the corresponding sequence accession numbers are reported in Appendix A, Table A1.

DNA Extraction, PCR Amplification and Sequencing

Three of the *C. gloeosporioides* isolates included in this study, isolated from symptomatic citrus hosts but previously uncharacterised, were subjected to molecular characterization. The isolates were cultured on PDA or malt extract agar (MEA, Oxoid, Basingstoke, UK) for seven days before genomic DNA extraction. Mycelium was scraped off and processed according to the respective manufacturer's instructions using the Wizard Genomic DNA Purification Kit® (Promega Corporation, Madison, WI, USA) for the Italian isolates (LA2 and LI3) and Plant/Fungi DNA Isolation Kit® (Norgen Biotek Corp., Thorold, ON, Canada) for the Spanish isolate (PV-1457). Beta-tubulin (*TUB2*), actin (*ACT*), chitin synthase 1 (*CHS-1*), glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) and the intergenic region of apn2 and MAT1-2 genes (*ApMat*) were the five genomic areas amplified and sequenced to confirm the identity of fungal isolates belonging to the *C. gloeosporioides* species complex. Primers and PCR conditions for each genomic region are listed in Table 3.

PCR products were examined on agarose gels and subsequently sequenced in both directions. Sequencing was performed by Macrogen Inc. (Seoul, Republic of Korea) for the Italian isolates (LA2 and LI3) and by STAB VIDA (Caparica, Portugal) for the Spanish isolate (PV-1457). The obtained sequences were viewed and manually edited using MEGA 11 (Molecular Evolutionary Genetics Analysis) [60]. BLASTn searches of the five loci were conducted using the NCBI (National Center for Biotechnology Information) BLAST website to identify the closest sequences (https://blast.ncbi.nlm.nih.gov/Blast.cgi, accessed on 10 January 2026). The BLASTn results are reported in Appendix A, Table A1, and all sequences obtained from the amplified genomic regions of all isolates were deposited in the GenBank database.

Table 3. Genomic regions analyzed, forward and reverse primers and PCR conditions for molecular characterization.

Genomic Region	Forward Primer	Reverse Primer	PCR Conditions	References
TUB2	Bt2a	Bt2b	1. Den [1]: 95 °C for 5 min, followed by 40 cycles of 95 °C for 15 s, 2. Ann [2]: 52 °C for 15 s and 72 °C for 10 s, 3. Ext [3]: 72 °C for 7 min.	[56]
ACT	ACT-512F	ACT-783R	1. Den [1]: 95 °C for 5 min, followed by 40 cycles of 95 °C for 15 s, 2. Ann [2]: 52 °C for 15 s and 72 °C for 10 s, 3. Ext [3]: 72 °C for 7 min.	[57]
CHS-1	CHS-354R	CHS-79F	1. Den [1]: 95 °C for 5 min, followed by 40 cycles of 95 °C for 15 s, 2. Ann [2]: 52 °C for 15 s and 72 °C for 10 s, 3. Ext [3]: 72 °C for 7 min.	[57]
GAPDH	GDF1	GDR1	1. Den [1]: 95 °C for 5 min, followed by 40 cycles of 95 °C for 15 s, 2. Ann [2]: 52 °C for 15 s and 72 °C for 10 s, 3. Ext [3]: 72 °C for 7 min.	[58]
ApMat	AMF1	AMR1	1. Den [1]: 95 °C for 5 min, followed by 40 cycles of 95 °C for 15 s, 2. Ann [2]: 55 °C for 15 s and 72 °C for 10 s, 3. Ext [3]: 72 °C for 7 min.	[59]

[1] Den: denaturation; [2] Ann: annealing; [3] Ext: extension.

2.3. In Vitro Antifungal Efficacy

2.3.1. Mycelial Growth Inhibition

A preliminary assay was performed to assess whether the seven selected nanoemulsified essential oils exhibited inhibitory or stimulatory effects. Accordingly, each N-EO was incorporated at 1% *v*/*v* into sterilized PDA and cooled to approximately 45 °C. The medium was gently stirred to ensure homogeneous distribution of the emulsion and then poured into 90 mm sterile Petri plates. Streptomycin sulfate (0.1 mg L^{-1}) ((Sigma-Aldrich, St. Louis, MO, USA)) was also added to the medium to prevent bacterial contamination. Mycelial plugs (5 mm in diameter) from the margin of 7-day-old fungal colonies were placed in the middle of each plate and incubated at 24 ± 2 °C for five days for *N. parvum* isolates and seven days for *Colletotrichum* spp. isolates. Antibiotic PDA supplemented with 5% *v*/*v* of Tween 80® served as the control, matching the emulsifier concentration used in all N-EO formulations (5% Tween 80®, 80% SDDW, 15% essential oil). The preliminary assay was arranged in a completely randomized design with three replicate plates per N-EO and isolate combination (7 N-EOs × 6 isolates × 3 replicates = 126 plates) and was performed once.

Following this first screening, all seven N-EOs were further examined in a subsequent assay using the same experimental conditions, but testing a set of five concentrations (0.1, 0.5, 1.0, 3.0 and 5.0% *v*/*v*). There were three replicate Petri dishes per N-EO and dose combination for each isolate (6 isolates in total). A factorial design with two independent factors (7 N-EOs and 5 doses per product) was used. Thus, the experiment included 6 × 7 × 5 × 3 = 630 Petri plates and was conducted twice (total = 1260 Petri plates). After incubation, two perpendicular colony diameters were measured, and mycelium growth inhibition (MGI; %) was calculated according to Guarnaccia et al. [61]:

$$MGI = [1 - d_t/d_c] \times 100, \quad (1)$$

where d_t and d_c represent the average colony diameter in the treated and control plates, respectively.

2.3.2. Conidial Germination Inhibition

PDA plates and Pistachio Leaf Agar (PLA) [62] plates were prepared as substrates for the cultivation of *C. gloeosporioides* and *N. parvum* isolates, respectively. After 10 days of incubation at 24 ± 2 °C, the colony surfaces were gently scraped, and the resulting material was filtered through a sterile gauze (ADA swabs) and the conidial suspension obtained was quantified using a hemacytometer. For the germination assay, both the conidial suspensions and the N-EOs solutions were prepared at double strength, as they were subsequently combined in equal volumes (1:1) to obtain the final working concentrations. Conidia were adjusted to 2×10^5 spores mL $^{-1}$ before mixing, resulting in a final concentration of 1×10^5 spores mL $^{-1}$ after combining with the N-EOs solutions. N-EOs dilutions were prepared at 0.2, 0.6, 1.8, 5.4, and 16.2% *v*/*v*, therefore producing final concentrations of 0.1, 0.3, 0.9, 2.7, and 8.1% *v*/*v*. A control solution consisting of sterile double-distilled water (SDDW) supplemented with 5% Tween 80® was prepared using the same procedure. Following the procedure described by Moral et al. [63], a 5 µL drop of the conidial suspension of each isolate and a 5 µL drop of each N-EOs concentration were placed in the middle of a microscope coverslip (20 × 20 mm). The coverslip was placed inside Petri dishes containing water agar, which are used as a humid chamber. Petri dishes were sealed with Parafilm® (Bemis Company Inc., Neenah, WI, USA) and incubated at 24 ± 2 °C in the dark for 5 h for *N. parvum* and 12–14 h for *C. gloeosporioides*. For each isolate (6 isolates), there were three replicate coverslips per N-EO and concentration. A factorial design with two independent factors (7 N-EOs and 5 doses per product) was used, and the entire experiment was conducted twice (resulting in 6 × 7 × 5 × 3 × 2 = 1260 coverslips). After the incubation period, 5 µL of 0.01% fuchsine acid in lactoglycerol (1:2:1 lactic acid/glycerol/water) was added to each coverslip to stop conidia germination. The percentage of spore germination was determined by randomly counting 100 conidia for each coverslip using a Nikon Eclipse 80i microscope with ×400 magnification (Nikon Corp., Tokyo, Japan). If the germ tube did not reach at least half of the longitudinal axis of the spore, the spore was not considered germinated. Then, the conidial germination inhibition (CGI; %) was calculated according to López-Moral et al. [64]:

$$CGI = [1 - Ge_t/Ge_c] \times 100, \quad (2)$$

where Ge_t and Ge_c represent the percentage of germination in the treatments and control, respectively.

2.4. In Vivo Antifungal Efficacy

2.4.1. Lemon Fruits Inoculated with *Colletotrichum gloeosporioides*

Lemon fruits (*Citrus limon* L. cv. Zagara Bianca) were harvested from an organic orchard to evaluate the *in vivo* antifungal activity of selected N-EOs, identified as highly effective *in vitro*, against two *C. gloeosporioides* isolates (one Italian and one Spanish), which are the causal agents of anthracnose, causing sunken necrotic lesions on fruit tissues [65]. Fruits were washed with a commercial detergent to remove surface impurities and subsequently surface-disinfected by immersion in 1.5% sodium hypochlorite for 10 min, followed by two rinses with sterile distilled water (SDW) [10]. After air-drying, three wounds per fruit were created using a sterile cork borer (5 mm diameter) to remove the exocarp. Wounded fruits were treated with clove, garlic, and lavender nanoemulsions. Lavender was applied at 5.0, 1.6, and 0.8% *v*/*v*, whereas garlic and clove were tested at 0.8, 0.4, and 0.2% *v*/*v*, as higher concentrations, like 5% and 1.6% *v*/*v*, caused phytotoxic effects in preliminary assays (Figure 1). Additionally, a treatment consisting of chemical fungicide (CABRIO®

WG; BASF SE, Ludwigshafen, Germany), applied at the maximum label-recommended dose, and a control consisting of Tween 80® at 5% *v*/*v* were included.

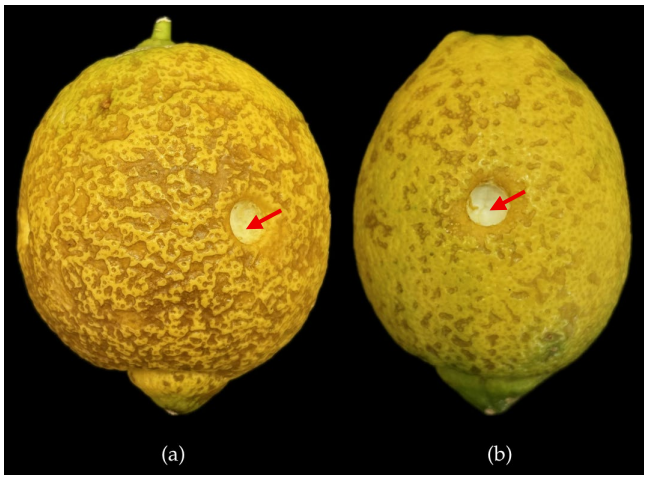


Figure 1. Phytotoxic effects observed on lemon fruits treated with clove N-EO: (**a**) 5.0% *v*/*v*; (**b**) 1.6% *v*/*v*. The cork borer wounds are visible (red arrows), as lesions were made before N-EO application to allow uniform spraying at the inoculation points.

Once the fruits had dried, a 5 mm mycelial plug from the margin of 7-day-old cultures of *C. gloeosporioides* (LI3: Italy, COL-69: Spain) grown on PDA as described above was placed onto each wound and sealed with Parafilm® to maintain local humidity. Then, inoculated fruits were placed in plastic boxes (≈40 × 60 × 13 cm) containing sterile perlite moistened (approximately 120 g) with sterile distilled water (500 mL) to maintain 100% relative humidity and incubated at 24 ± 2 °C in the dark. For each isolate (2 isolates), a completely randomized design was used with treatment as the independent factor, including nine N-EO combinations (3 N-EOs × 3 concentrations), the chemical fungicide and the control (11 treatments in total). For each treatment, four lemons were used, each with three inoculation points. All fruits belonging to the same treatment were incubated together in the same plastic box (11 × 4 = 44 lemons and 11 × 4 × 3 = 132 inoculation sites). The experiment was conducted twice (total per isolate = 88 lemons and 264 inoculation sites). The two orthogonal diameters were measured 10 days after the inoculation in both control and treated lemons, and the mean orthogonal diameters of the lesions were calculated.

2.4.2. Lemon Twigs Inoculated with *Neofusicoccum parvum*

Similarly, detached twigs of *Citrus* × *volkameriana*, a widely used lemon rootstock in Sicily and known to be susceptible to *N. parvum* [19], were collected to evaluate the *in vivo* antifungal activity of selected N-EOs, identified as highly effective *in vitro*. One-year-old lignified but still green twigs were cut to approximately 20 cm and surface-sterilized by sequential immersion in 70% ethanol (30 s), sodium hypochlorite (1 min), and 70% ethanol (30 s), following the protocol described by Catalano et al. [66]. Sterilized twigs were left to air-dry under a laminar-flow hood, and both ends were sealed with pruning wax. After wax solidification, wounds were made at the midpoint of each twig with a sterile needle and marked for reference. The entire surface of the twigs was then uniformly sprayed with the selected N-EO treatments. The nanoemulsions tested were clove, garlic, and lavender, each applied at three concentrations (1.6, 0.8, and 0.2% *v*/*v*). Higher concentrations were not tested due to visible phytotoxic effects on the twig surface during preliminary trials. After the twigs had completely dried, a 5 mm mycelial plug from a 7-day-old *N. parvum* (AGR-CT-84 isolate) culture grown on PDA was placed upside down, with the mycelial surface in contact with the wound. Inoculated twigs were incubated in plastic boxes

(≈20 × 15 × 8 cm) containing sterile perlite moistened (approximately 20 g) with sterile distilled water (200 mL) to maintain 100% relative humidity and incubated under the same conditions described for the lemon fruits assay. Similar to the lemon fruit assay, treatments included nine N-EO combinations (3 N-EOs × 3 concentrations), a chemical fungicide (CABRIO® WG at the maximum label-recommended dose) and a control (Tween 80® at 5% *v*/*v*), for a total of 11 treatments. The experiment followed a completely randomized design with treatments (oil × dose) as independent factors. For each treatment, two plastic boxes were prepared as replicates, each containing four twigs with one inoculation site per twig (11 × 2 × 4 = 88 twigs and 88 inoculation sites per assay). The experiment was conducted twice (total = 176 twigs and 176 inoculation sites). Lesion development was assessed by measuring lesion length 7 days post-inoculation in both control and treated twigs. Finally, the mean lengths of the lesions were calculated.

2.5. Data Analysis

As both the *in vitro* and *in vivo* experiments were performed twice, normality and homogeneity of variance were checked, and because no significant differences were detected, the data were pooled for subsequent analyses. For *in vitro* assays, mycelial growth inhibition (MGI; %) and conidial germination inhibition (CGI; %) data were used to estimate the concentrations required to inhibit 50% and 90% of mycelial growth (EC_{50} and EC_{90}). MGI or CGI data were regressed against dose (or log-transformed dose), and dose–response curves were fitted using non-linear regression models. The best-fit model was selected based on the highest coefficient of determination (R^2) among the three-parameter exponential, three-parameter Gompertz and four-parameter logistic models. A factorial ANOVA was conducted using EC_{50} and EC_{90} as dependent variables and N-EO, isolate, and their interaction (N-EO × isolate) as independent factors. Because the N-EO × isolate interaction was significant in both cases ($p \leq 0.0001$), simple effects were explored by conducting separate one-way ANOVAs. First, isolate was used as the independent factor within each N-EO to assess variability among isolates for the same oil; then, N-EO was used as the independent factor within each isolate to compare oil performance for the same isolate. When significant effects were detected, means were compared using Fisher's LSD test at $p = 0.05$ [67].

For the *in vivo* trials conducted on lemon fruits and detached twigs, data for each isolate were analyzed separately by one-way ANOVA. Mean orthogonal diameters (fruit assay) or lesion length (twig assay) were used as dependent variables, and each N-EO × concentration combination (e.g., garlic–max, garlic–medium, garlic–min) as independent variables. As stated above, means were compared using Fisher's least significant difference (LSD) test at $p = 0.05$ [67] if significant differences were detected. All statistical analyses were performed using Statistix 10 software [68].

3. Results

3.1. Physical Characterization of Formulated N-EOs

After 24 h of formulation, the particle size (nm), polydispersity index (PDI), and zeta potential (mV) were determined for each nanoemulsion as shown in Table 4. All the N-EOs exhibited droplet sizes below 200 nm, confirming their nanometric range and the efficiency of the emulsification process. Significant differences in particle size were observed among the formulations ($p < 0.05$). Citronella N-EO significantly showed the smallest droplet size (103.8 ± 1.83 nm). In contrast, clove and peppermint N-EOs exhibited the largest droplet sizes (150.43 ± 1.25 and 146.43 ± 2 nm, respectively) and did not differ significantly from each other. The polydispersity index (PDI) ranged from 0.12 ± 0.003 to 0.194 ± 0.003, with all values below 0.2, indicating good homogeneity. However, significant differences among formulations were observed ($p < 0.05$). Clove and fennel N-EOs exhibited the highest

PDI values, suggesting greater heterogeneity in droplet size distribution, whereas citronella, lavender and peppermint showed lower values. Notably, laurel exhibited the lowest PDI value, differing significantly from all formulations and indicating the highest homogeneity. The zeta potential values ranged between −16.9 mV and −32.7 mV, with significant differences among formulations ($p < 0.05$). The lowest values were observed for citronella (−32.7 ± 2.37 mV) and clove (−29.83 ± 0.60 mV), suggesting enhanced electrostatic stabilization. In contrast, laurel, lavender, and peppermint exhibited higher zeta potential values and were statistically similar, indicating potentially lower long-term stability.

Table 4. Physical characteristics (size, PDI, and zeta potential) of the N-EOs measured 24 h after formulation.

N-EOs [1]	Size (nm)	PDI [2]	Zeta Potential (mV)
Citronella	103.8 ± 1.83 d	0.142 ± 0.006 c	−32.73 ± 2.37 c
Clove	150.43 ± 1.25 a	0.1943 ± 0.003 a	−29.83 ± 0.60 c
Fennel	120.63 ± 0.87 b	0.1933 ± 0.007 a	−22.57 ± 0.61 b
Garlic	124.13 ± 6.35 b	0.1647 ± 0.004 b	−19.93 ± 1.02 ab
Laurel	112.87 ± 1.76 c	0.12 ± 0.003 d	−16.9 ± 0.2 a
Lavender	123.27 ± 0.25 b	0.1397 ± 0.002 c	−18.57 ± 0.42 a
Peppermint	146.43 ± 2 a	0.1493 ± 0.001 c	−19.17 ± 0.93 a
F; df; *p* level	113, 24; 6; <0.001	126.78; 6; <0.001	91.77; 6; <0.001

Data represent mean values from three independent replicates ± standard deviation. Different letters denote statistically significant differences among the N-EOs for each physical characteristic ($p < 0.05$; one-way ANOVA; Tukey's post hoc test). [1] Nanoemulsion based on essential oils. [2] Polydispersity Index.

3.2. In Vitro Antifungal Efficacy

3.2.1. Mycelial Growth Inhibition

From the preliminary assay in which all N-EOs were tested at 1% *v*/*v*, it was found that all inhibited the growth of the pathogens. Consequently, all seven oils were tested at the aforementioned five concentrations. EC_{50} values of mycelial inhibition for both fungal species are presented in Figure 2.

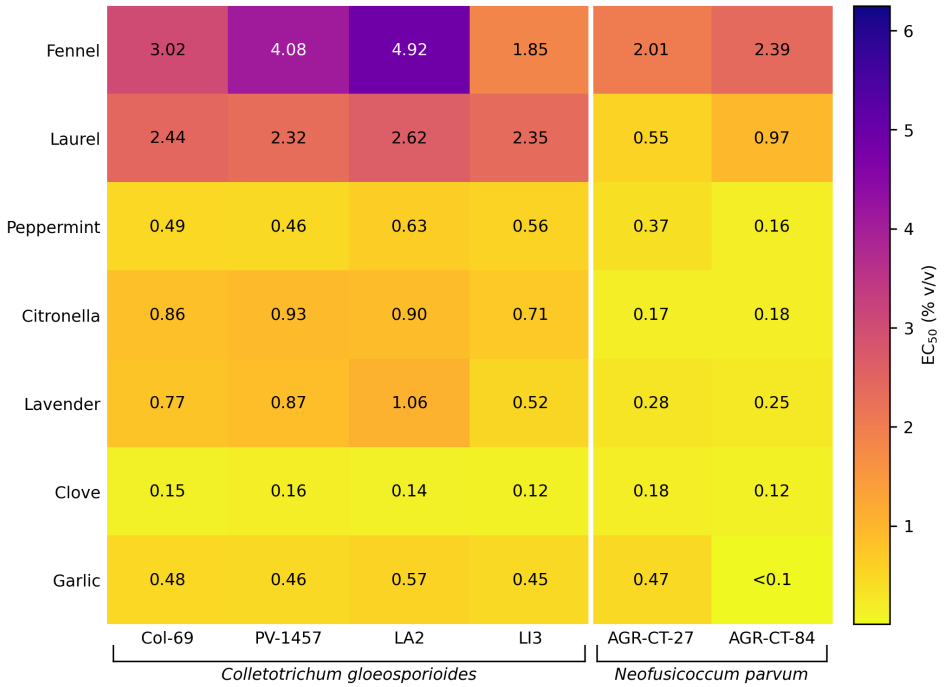


Figure 2. Graphical visualization of EC_{50} values (% *v*/*v*) of each nanoemulsion based on essential oils (N-EOs) inhibiting mycelial growth of *Colletotrichum gloeosporioides* (Col-69, PV-1457, LA2 and LI3 isolates) and *Neofusicoccum parvum* (AGR-CT-27 and AGR-CT-84 isolates).

Colletotrichum gloeosporioides

For each N-EO, variability across isolates was assessed (Appendix B, Table A2) and was pronounced for several oils, whereas others revealed more uniform results. In detail, observing the EC_{50} values, fennel and lavender showed the highest variability among isolates ($p \leq 0.0001$), whereas laurel and citronella did not show significant differences. For example, fennel was significantly less effective for LA2 (4.92%) than for Col-69 (3.02%) and LI3 (1.85%), and lavender differed markedly, showing its highest EC_{50} for LA2 and its lowest for LI3. Also, garlic displayed a similar pattern, with greater efficacy for LI3 and PV-1457 than for LA2.

Within each isolate, oil efficacy differed significantly ($p \leq 0.0001$), but overall, it remained largely uniform. As shown in Figure 2, clove was consistently the most effective treatment for all the *C. gloeosporioides* isolates tested, showing the lowest EC_{50} values (0.12–0.16% v/v), followed by garlic and peppermint. Citronella and lavender generally showed comparable and intermediate efficacy. In contrast, fennel and laurel significantly displayed the lowest efficacy for all isolates, with fennel reaching the highest EC_{50} values. A similar pattern was observed for EC_{90} values in Appendix B, Table A2. Representative Petri plates showing the antifungal activity of N-EOs against *C. gloeosporioides* (LI3) are shown in Figure 3.

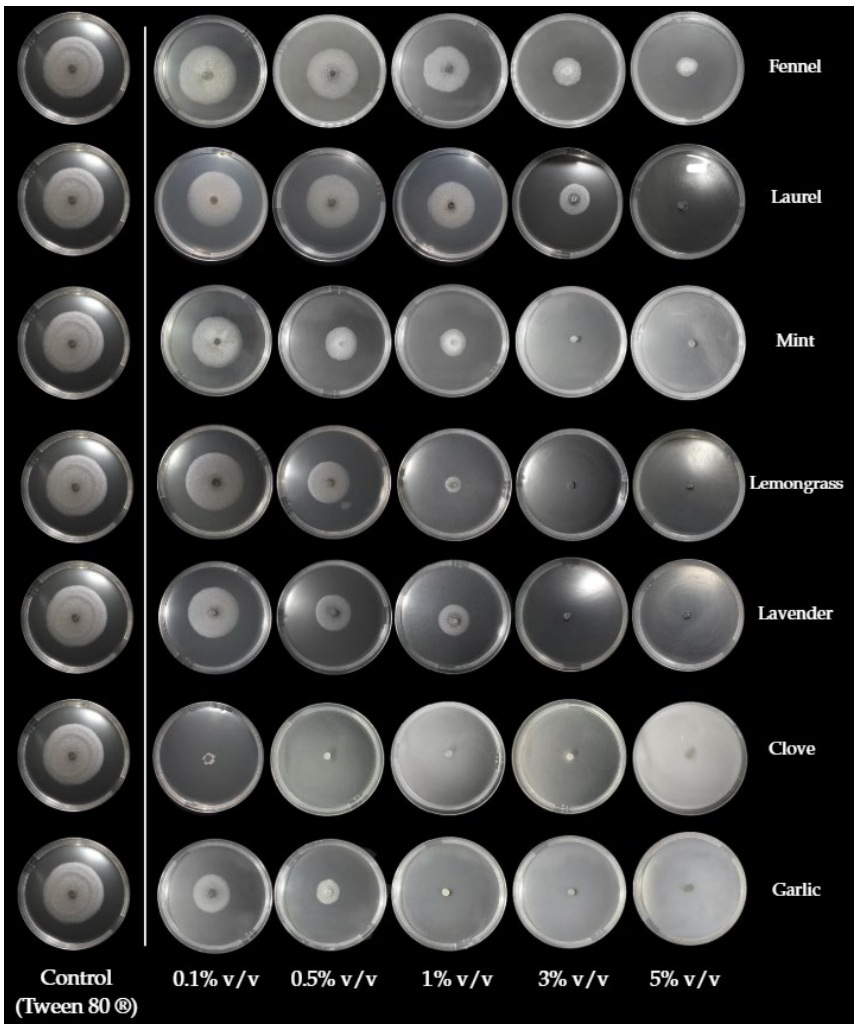


Figure 3. Representative Petri plates showing the antifungal activity of seven N-EOs tested at five different concentrations against *Colletotrichum gloeosporioides* (LI3) after seven days of incubation at 24 °C. For each nanoemulsion, fungal growth is compared with the control (Tween 80®).

Neofusicoccum parvum

As regards EC_{50} values for *N. parvum*, significant differences were found among isolates for laurel, peppermint and garlic ($p \leq 0.0001$; Appendix B, Table A3). Garlic exhibited the strongest variability effect, with AGR-CT-84 being markedly more sensitive than AGR-CT-27. In contrast, fennel, citronella, clove and lavender N-EOs showed similar EC_{50} values across isolates.

N-EOs efficacy within each isolate differed significantly ($p \leq 0.0001$) and showed both shared and isolate-specific patterns, as illustrated in Figure 2. Fennel was the least effective treatment for both isolates, with values of EC_{50} = 2.01% observed in AGR-CT-27 and EC_{50} = 2.39% in AGR-CT-84, significantly differing from the most active oils and followed by laurel N-EO. In AGR-CT-27 isolate, the most effective oils were citronella and clove (EC_{50} = 0.17 and 0.18%, respectively), followed by lavender, peppermint, and then garlic. In AGR-CT-84 isolate, the overall pattern was similar for most treatments, although garlic showed significantly greater efficacy, resulting in complete inhibition at the lowest tested concentration (EC_{50} < 0.1%, total inhibition at 0.1% *v*/*v*). Representative Petri plates showing the antifungal activity of N-EOs against *N. parvum* (AGR-CT-84) are shown in Figure 4. Collectively, EC_{50} values for *N. parvum* were lower than those observed for *C. gloeosporioides*, suggesting a potentially greater sensitivity of this pathogen to the tested N-EOs.

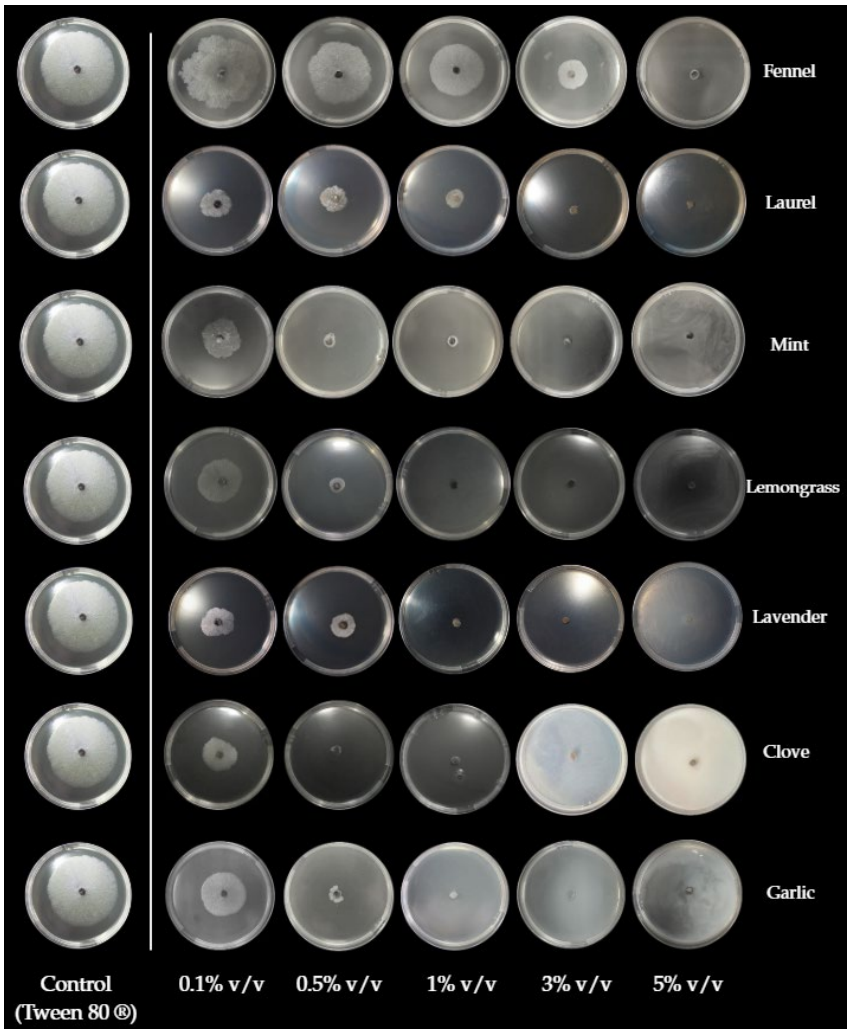


Figure 4. Representative Petri plates showing the antifungal activity of seven N-EOs tested at five different concentrations against *Neofusicoccum parvum* (AGR-CT-84) after five days of incubation at 24 °C. For each nanoemulsion, fungal growth is compared with the control (Tween 80®).

3.2.2. Conidial Germination Inhibition

Similarly to the previous *in vitro* assay, EC_{50} values of conidial germination inhibition for both pathogens are presented in Figure 5.

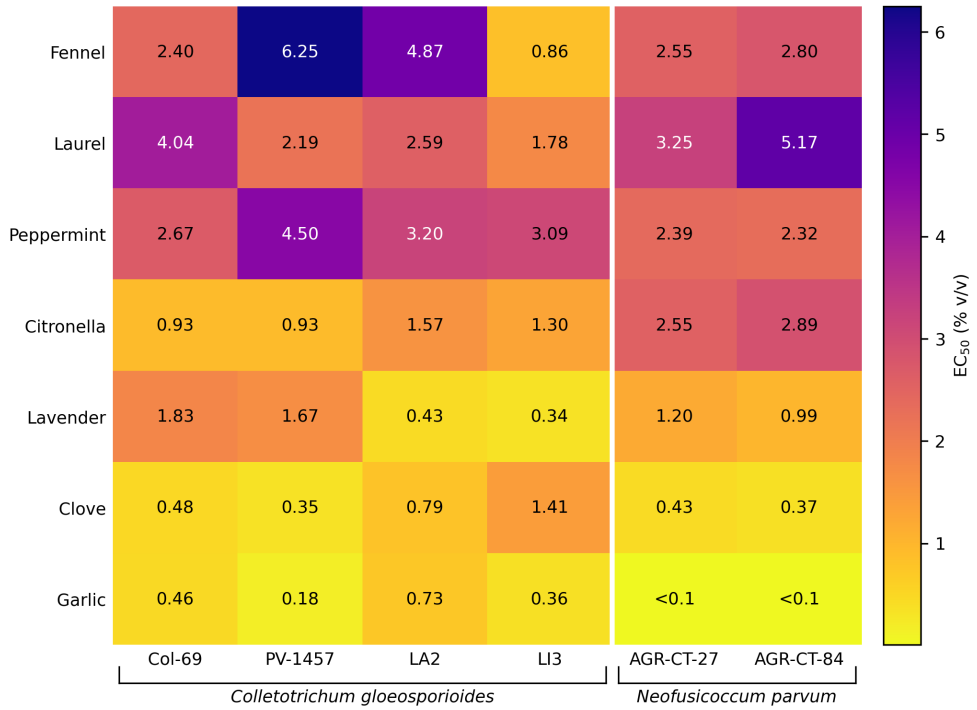


Figure 5. Graphical visualization of EC_{50} values (% *v*/*v*) of each nanoemulsion based on essential oils (N-EOs) inhibiting conidial germination of *Colletotrichum gloeosporioides* (Col-69, PV-1457, LA2 and LI3 isolates) and *Neofusicoccum parvum* (AGR-CT-27 and AGR-CT-84 isolates).

Colletotrichum gloeosporioides

In the conidial germination assay against *C. gloeosporioides*, the variability among isolates within the same N-EO strongly depended on the oil tested (Appendix B, Table A4). Fennel once again showed the greatest difference between isolates (EC_{50} = 0.86−6.25%), as well as for garlic. Laurel and clove exhibited intermediate differences between isolates, similar to citronella, lavender and peppermint.

Oil performance strictly depended on the isolate considered (Figure 5). Specifically, considering Col-69, clove, and garlic significantly differed ($p < 0.001$) from the other treatments, similarly for PV-1457. For LA2 isolate, lavender was also ranked among the most promising treatments, followed by garlic and clove, with no significant differences among the three treatments. Similar results were observed for LI3, except for clove, which presented EC_{50} values comparable to those of laurel. In contrast to the results obtained for mycelial growth, peppermint was among the least effective N-EOs, together with fennel and laurel. These nanoemulsions showed significant differences from other N-EOs, exhibiting higher EC_{50} values, particularly in PV-1457 (fennel: EC_{50} = 6.25%; peppermint: EC_{50} = 4.50%) and LA2 (fennel: EC_{50} = 4.87%; peppermint: EC_{50} = 3.20%). Similar patterns are shown for EC_{90} values in Appendix B, Table A4.

Neofusicoccum parvum

In the conidial germination assay against *N. parvum*, variability between isolates was not observed within the same oil, except for laurel and citronella, which showed higher EC_{50}/EC_{90} values for AGR-CT-84 than for AGR-CT-27 (Appendix B, Table A5).

As regards treatment efficacy, garlic N-EO was the most effective in both isolates (Figure 5), leading to total inhibition at the lowest concentration tested (EC_{50} < 0.1%). Once

again, clove showed promising results, followed by lavender. Although fennel, peppermint, and citronella N-EOs exhibited relatively high EC_{50} values, laurel was clearly the least effective treatment, differing significantly from all the others.

3.3. In Vivo Antifungal Efficacy

3.3.1. Antifungal Efficacy on Lemon Fruits Against *Colletotrichum gloeosporioides*

Italian *Colletotrichum gloeosporioides*

The *in vivo* experiment performed on lemons inoculated with the LI3 isolate of *C. gloeosporioides* (Figure 6) largely supported the trends previously observed *in vitro* and displayed significant differences among treatments ($p \leq 0.0001$).

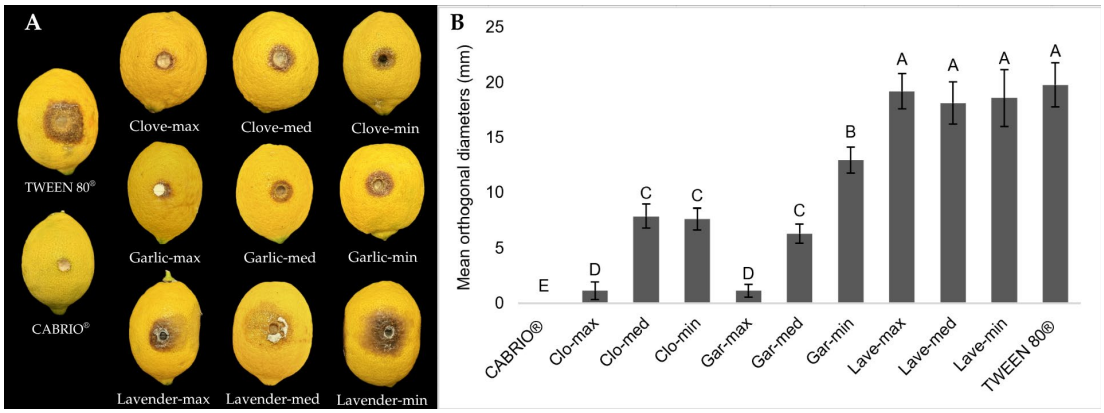


Figure 6. (**A**): Representative lemon fruits inoculated with *Colletotrichum gloeosporioides* (LI3 isolate) showing differences in lesion size among treatments, incubated for ten days at 24 °C in the dark. Clove, garlic and lavender were tested at three concentrations (max, med, min). CABRIO® represents the chemical treatment, and Tween 80® the control. (**B**): Effect of selected N-EOs on lesion development of *Colletotrichum gloeosporioides* (LI3 isolate) on lemon fruits. Bars show the mean of the two orthogonal diameters from four replicate lemon fruits ± SE. Different letters indicate significant differences among treatments (Fisher's LSD, $p \leq 0.05$).

The control (Tween 80®) resulted in the largest lesion, while the chemical treatment (CABRIO® WG) completely inhibited lesion development. Clove and garlic, when applied at the maximum concentration, were the most effective treatments and differed significantly from all the others. These treatments showed dose-dependent efficacy, with lower concentrations showing reduced efficacy. Whereas lavender N-EO did not differ from the control at the three tested concentrations, highlighting the difficulty of translating laboratory efficacy to *in vivo* fruit infection conditions, where interactions with host tissues and environmental factors could have influenced treatment performance.

Spanish *Colletotrichum gloeosporioides*

Similar to LI3 isolate, lesion development significantly differed among treatments ($p \leq 0.0001$; Figure 7) for Col-69 isolate.

Once again, lemons treated with the Tween 80® (control) showed the largest lesion development (20.5 ± 0.8 mm), and the chemical treatment (CABRIO® WG) completely inhibited lesion development. Garlic N-EO was the most effective at the maximum and medium concentrations, displaying the smallest lesions (9.02 ± 0.9 and 9.54 ± 0.8 mm), whereas no efficacy was observed at the minimum concentration. Clove significantly reduced the lesion diameters only at the maximum concentration (13.21 ± 0.8 mm), although no efficacy was observed at the medium and minimum concentrations (17.50 ± 1.3 and 17.13 ± 0.8 mm, respectively). Similar values were observed for lavender at maximum and medium concentrations (18.9 ± 2.7 mm and 18.8 ± 2.1 mm), confirming the lowest

in vivo efficacy. Although the minimum concentration demonstrated slightly improved performance (14.83 ± 1.0 mm), this was insufficient to provide reliable protection.

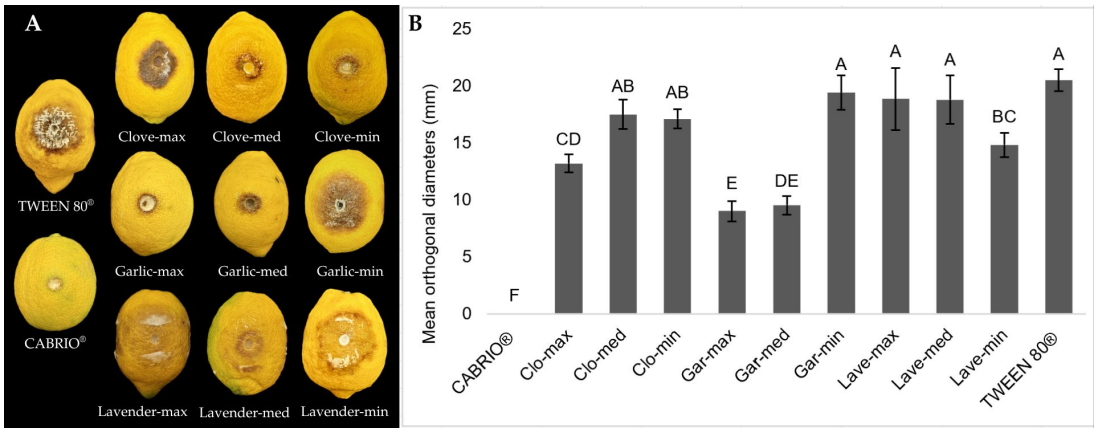


Figure 7. (**A**): Representative lemon fruits inoculated with *Colletotrichum gloeosporioides* (Col-69 isolate), showing differences in lesion size among treatments, incubated for ten days at 24 °C in the dark. Clove, garlic and lavender were tested at three concentrations (max, med, min). CABRIO® represents the chemical treatment, and Tween 80® the control. (**B**): Effect of selected N-EOs on lesion development of *Colletotrichum gloeosporioides* (Col-69 isolate) on lemon fruits. Bars show the mean of the two orthogonal diameters from four replicate lemon fruits ± SE. Different letters indicate significant differences among treatments (Fisher's LSD, $p \leq 0.05$).

3.3.2. Antifungal Efficacy on Lemon Twigs Against *Neofusicoccum parvum*

As stated above, significant differences ($p < 0.0001$; Figure 8A) were observed among treatments on detached lemon twigs inoculated with *N. parvum*. Garlic applied at the maximum and medium concentrations (1.6 and 0.8% v/v) induced heavy caustic effects on twig tissues (Figure 8B), limiting its suitability for potential field applications. As a consequence, only the lowest concentration (0.2% v/v) was included in the statistical analysis. Even at this dosage, it significantly reduced lesion length (4.9 ± 0.9 mm) compared with the control, which showed the largest lesions (23.5 ± 1.8 mm). Similar efficacy was observed for clove at maximum concentration (5.7 ± 0.5 mm), thus confirming its strong antifungal activity.

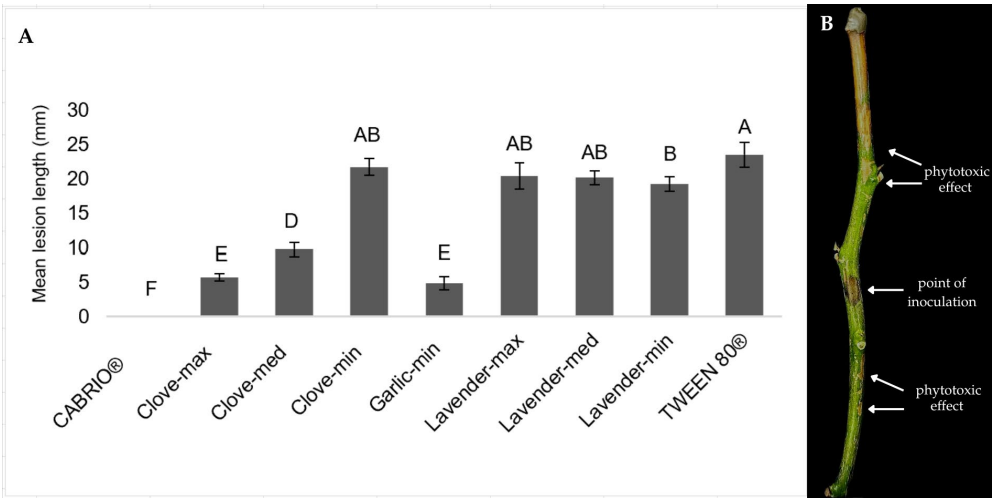


Figure 8. (**A**): Mean lesion length (mm) on detached twigs inoculated with *Neofusicoccum parvum* (AGR-CT-84 isolate) after N-EO treatments. Clove and lavender were tested at 1.6, 0.8, and 0.2% v/v; garlic at 0.2% v/v. CABRIO® represents the chemical treatment, and Tween 80® the control. Bars represent mean ± SE. Different letters indicate significant differences (Fisher's LSD, $p \leq 0.05$). (**B**): Phytotoxic effects observed on lemon twigs treated with garlic N-EO (1.6% v/v).

Intermediate efficacy was detected when clove was applied at the medium concentration (9.7 ± 0.9 mm), whereas the minimum concentration (21.7 ± 1.2 mm) did not differ significantly. Similarly, lavender was largely ineffective, with the maximum and medium concentrations (20.5 ± 1.9 mm and 20.1 ± 1.0 mm, respectively) being comparable to the control, and only a slight reduction was observed at the minimum concentration (19.2 ± 1.1 mm). Representative lemon twigs inoculated with AGR-CT-84 isolate are shown in Figure 9.



Figure 9. Representative lemon detached twigs inoculated with *Neofusicoccum parvum* (AGR-CT-84 isolate), showing differences among treatments in lesion size, seven days after incubation at 24 °C.

4. Discussion

The reduction in chemicals represents a key priority in modern agriculture, due to their documented impacts on human health and the environment [24]. The implementation of alternative control strategies based on biological or naturally derived compounds is therefore increasingly encouraged. Within this framework, essential oil-based nanoemulsions (N-EOs) have emerged as potential biopesticides, combining the antifungal properties of essential oils with enhanced physicochemical stability and bioavailability of the nanoformulations [53]. This study highlights the potential antifungal activity of seven N-EOs, particularly citronella, clove, fennel, garlic, laurel, lavender, and peppermint, against *C. gloeosporioides* and *N. parvum*, both *in vitro* and *in vivo*. Although the antifungal activity of essential oils has been widely investigated against *Colletotrichum* spp. [26,27,31–33,36,39], only a limited number of studies have focused on *N. parvum* [37,49], most of which evaluated pure oils *in vitro* rather than their nanoemulsion formulation. To the best of our knowledge, this is the first research evaluating nanoemulsion formulations containing the aforementioned essential oils against *C. gloeosporioides* and *N. parvum*.

In vitro screening revealed that all seven essential oils tested reduced mycelial growth and conidial germination of the fungal isolates tested, although their efficacy varied depending on the oil, pathogen and, in some cases, the isolate. No consistent grouping based on geographical origin was detected among *C. gloeosporioides* isolates from Spain (Col-69, PV-1457) and Italy (LA2, LI3), indicating that differences between isolates primarily depended on the N-EO tested and the assay (mycelial growth or conidial germination). Similarly, the differences observed among *N. parvum* isolates were limited and dependent on the N-EOs. However, for *N. parvum*, lower EC_{50}/EC_{90} values were generally observed in the MGI, indicating that smaller amounts of N-EO were necessary to achieve compa-

rable inhibition levels to those observed for *C. gloeosporioides*. As regards the efficacy of treatments, consistent and reproducible patterns emerged across fungal species. Clove and garlic consistently exhibited the lowest EC_{50} values in both assays, whereas fennel and laurel were generally the least effective, and lavender, citronella, and peppermint displayed intermediate activity. The superior performance of clove and garlic is probably related to their major bioactive compounds, such as eugenol in clove [69] and organosulfur compounds, such as allicin in garlic [70], which are known to exert multiple antifungal effects. In this context, the mode of action associated with the essential oils is variable, from the disruption of cell membranes to compromising metabolic processes and inducing oxidative stress [71]. Moreover, the low efficacy of fennel and laurel suggests that their chemical profiles are less suitable for controlling these pathogens under the conditions tested.

Based on the *in vitro* results, garlic and clove, which showed the highest antifungal efficacy, together with lavender, which displayed intermediate efficacy, were selected for *in vivo* evaluation. These assays partially confirmed the *in vitro* screening, as garlic and clove again significantly reduced lesion development on both lemon fruits and detached lemon twigs, whereas lavender showed poor efficacy. Since this is the first evaluation of N-EOs on lemon fruits and twigs, the discrepancies observed between *in vitro* and *in vivo* performance should be interpreted with caution. Indeed, the reduced efficacy under *in vivo* conditions may reflect the greater complexity of host tissues, where physical and chemical characteristics of the fruit or twig surface can influence the behavior of the applied formulations. In citrus fruits, for example, endogenous essential oils and volatile compounds are mainly localized in the flavedo [72], and they may potentially interact with applied N-EOs in a synergistic or antagonistic way, influencing compound penetration, persistence, or bioavailability at the infection site [73,74]. Furthermore, higher N-EOs concentrations did not necessarily result in stronger antifungal effects. In this context, the phytotoxic effects observed at higher doses may partially damage host tissues, potentially hiding antifungal activity despite the intrinsic efficacy of the compounds against pathogenic fungi [75]. This interpretation is consistent with previous evidence for several essential oils, including eugenol, the main bioactive component of clove, whose activity has been associated with both antifungal and phytotoxic effects depending on concentration [76]. Moreover, the high volatility of essential oils represents a further limitation for practical application, as rapid evaporation may reduce persistence and bioavailability at the infection site.

Comprehensively, this study demonstrates that nanoemulsified essential oils, particularly those based on clove and garlic, can provide consistent antifungal activity against the selected citrus pathogens under both *in vitro* and *in vivo* conditions. In contrast, lavender displayed limited efficacy. The results highlight the importance of considering pathogen variability and host tissue interactions when translating laboratory findings into practical applications. Although the outcomes support the potential of N-EOs as a sustainable alternative for citrus disease control, further research is needed to optimize formulations and application strategies, ensure host tissue compatibility and validate their effectiveness under field conditions.

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Data Availability Statement: The original contributions presented in this study are included in the article. Further inquiries can be directed to the corresponding authors.

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Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

N-EO	Essential Oil-Based Nanoemulsion
ANOVA	Analysis of Variance
LSD	Least Significant Difference

Appendix A

Table A1. Blastn results of the sequenced isolates.

Locus	Isolate ID	Genbank Accession Numbers	Reference Specimen [x]	Genbank [y]	Identity %
TUB2	LA2	PZ024116	*Colletotrichum gloeosporioides* gnqcly2	KC293626.1	99.55
	LI3	PZ024117			99.55
	PV-1457	PZ024118			100
ACT	LA2	PZ024122	*C. gloeosporioides* CFCC	PP424990.1	100
	LI3	PZ024123			100
	PV-1457	PZ024124			100
CHS-1	LA2	PZ024125	*C. gloeosporioides* 179	OR282947.1	97.09
	LI3	PZ024126			97.09
	PV-1457	PZ024127			100
GAPDH	LA2	PZ024119	*C. gloeosporioides* UMC013	MW081164.1	100
	LI3	PZ024120	*C. gloeosporioides* LGMF1454	KX059224.1	100
	PV-1457	PZ024121	*C. gloeosporioides* UMC013	MW081164.1	100
ApMat	LA2	PZ052639	*C. gloeosporioides* LF318	KJ954541.1	99.76
	LI3	PZ052640			99.88
	PV-1457	PZ052641			98.78

[x] isolate used as reference in BLAST comparisons; [y] accession numbers of reference specimen.

Appendix B

Table A2. EC_{50} and EC_{90} values (% *v*/*v*) of nanoemulsified essential oils (N-EOs) inhibiting the mycelial growth of *Colletotrichum gloeosporioides* isolates (Col-69, PV-1457, LA2 and LI3).

N-EOs	EC_{50} Values (% *v*/*v*)			
	Col-69	**PV-1457**	**LA2**	**LI3**
Fennel	3.02 ± 0.03 a/C	4.08 ± 0.09 a/B	4.92 ± 0.13 a/A	1.85 ± 0.02 b/D
Laurel	2.44 ± 0.06 b/A	2.32 ± 0.14 b/A	2.62 ± 0.15 b/A	2.35 ± 0.03 a/A
Peppermint	0.49 ± 0.02 e/BC	0.46 ± 0.02 d/C	0.63 ± 0.01 d/A	0.56 ± 0.03 d/B
Citronella	0.86 ± 0.02 c/A	0.93 ± 0.04 c/A	0.9 ± 0.01 c/A	0.71 ± 0.02 c/B
Lavender	0.77 ± 0.03 d/C	0.87 ± 0.04 c/B	1.06 ± 0.01 c/A	0.52 ± 0.003 d/D
Clove	0.15 ± 0.001 f/B	0.16 ± 0.005 e/A	0.14 ± 0.0 e/B	0.12 ± 0.0 f/C
Garlic	0.48 ± 0.01 e/B	0.46 ± 0.02 d/B	0.57 ± 0.007 d/A	0.45 ± 0.008 e/B
p-value	≤0.0001	≤0.0001	≤0.0001	≤0.0001
	EC_{90} values (% *v*/*v*)			
	Col-69	**PV-1457**	**LA2**	**LI3**
Fennel	7.65 ± 0.13 a/B	7.93 ± 0.05 a/AB	7.86 ± 0.29 a/AB	8.71 ± 0.48 a/A
Laurel	4.72 ± 0.04 b/A	4.59 ± 0.07 b/A	4.63 ± 0.06 b/A	4.58 ± 0.01 b/A
Peppermint	1.86 ± 0.09 cd/A	1.84 ± 0.1 c/A	1.26 ± 0.05 de/B	1.91 ± 0.17 c/A
Citronella	2.01 ± 0.01 c/A	2.1 ± 0.12 c/A	2.3 ± 0.17 d/B	1.63 ± 0.3 c/A
Lavender	1.8 ± 0.06 d/C	2.04 ± 0.09 c/B	2.35 ± 0.07 c/A	0.59 ± 0.01 d/D
Clove	0.19 ± 0.003 f/A	0.18 ± 0.003 e/A	0.18 ± 0.003 f/A	0.16 ± 0 d/B
Garlic	0.95 ± 0.03 e/AB	0.87 ± 0.09 d/B	1.03 ± 0.02 e/A	0.55 ± 0.003 d/C
p-value	≤0.0001	≤0.0001	≤0.0001	≤0.0001

Data present the mean of three replicates from two replicated experiments ± standard error; Means followed by common lowercase letters in a column, or by uppercase letters in a row, do not differ significantly according to Fisher's protected LSD test ($p = 0.05$).

Table A3. EC_{50} and EC_{90} values (% *v*/*v*) of nanoemulsified essential oils (N-EOs) inhibiting the mycelial growth of *Neofusicoccum parvum* isolates (AGR-CT-27 and AGR-CT-84).

N-EOs	EC_{50} Values (% *v*/*v*)	
	AGR-CT-27	**AGR-CT-84**
Fennel	2.01 ± 0.05 a/A	2.39 ± 0.14 a/A
Laurel	0.55 ± 0.1 b/B	0.97 ± 0.1 b/A
Peppermint	0.37 ± 0.003 cd/A	0.16 ± 0.01 cd/B
Citronella	0.17 ± 0.03 e/A	0.18 ± 0.02 cd/A
Lavender	0.28 ± 0.006 de/A	0.25 ± 0.05 c/A
Clove	0.18 ± 0.003 e/A	0.16 ± 0.0 cd/A
Garlic	0.47 ± 0.003 bc/A	<0.1 [x] d/B
p-value	≤0.0001	≤0.0001
	EC_{90} values (% *v*/*v*)	
	AGR-CT-27	**AGR-CT-84**
Fennel	4.94 ± 0.36 a/A	4.72 ± 0.18 a/A
Laurel	2.35 ± 0.39 b/A	1.87 ± 0.42 b/A
Peppermint	0.5 ± 0.0 c/A	0.52 ± 0.01 c/A
Citronella	0.56 ± 0.05 c/A	0.61 ± 0.02 c/A
Lavender	0.65 ± 0.03 c/A	0.66 ± 0.05 c/A
Clove	0.21 ± 0.003 c/A	0.20 ± 0.003 c/A
Garlic	0.55 ± 0.005 c/A	0.47 ± 0.003 c/A
p-value	≤0.0001	≤0.0001

Data present the mean of three replicates from two replicated experiments ± standard error ± standard error. Means followed by common lowercase letters in a column, or by uppercase letters in a row, do not differ significantly according to Fisher's protected LSD test ($p = 0.05$). [x] Indicate a total inhibition at the lower concentration tested (0.1% *v*/*v*).

Table A4. EC_{50} and EC_{90} values (% *v*/*v*) of nanoemulsified essential oils (N-EOs) inhibiting the conidial germination of *Colletotrichum gloeosporioides* isolates (Col-69, PV-1457, LA2 and LI3).

N-EOs	EC_{50} Values (% *v*/*v*)			
	Col-69	**PV-1457**	**LA2**	**LI3**
Fennel	2.4 ± 0.27 b/C	6.25 ± 0.01 a/A	4.87 ± 0.24 a/B	0.86 ± 0.003 d/D
Laurel	4.04 ± 0.49 a/C	2.19 ± 0.26 c/C	2.59 ± 0.03 c/B	1.78 ± 0.04 b/A
Peppermint	2.67 ± 0.01 b/C	4.5 ± 0.11 b/A	3.20 ± 0.29 b/B	3.09 ± 0.05 a/BC
Citronella	0.93 ± 0.007 d/B	0.93 ± 0.04 e/B	1.57 ± 0.006 d/A	1.30 ± 0.16 c/A
Lavender	1.83 ± 0.05 c/A	1.67 ± 0.6 d/A	0.43 ± 0.006 e/B	0.34 ± 0.0 e/B
Clove	0.48 ± 0.17 e/C	0.35 ± 0.005 f/C	0.79 ± 0.03 e/B	1.41 ± 0.003 c/A
Garlic	0.46 ± 0.02 e/B	0.18 ± 0.002 f/D	0.73 ± 0.05 e/A	0.36 ± 0.003 e/C
p-value	≤0.0001	≤0.0001	≤0.0001	≤0.0001
	EC_{90} values (% *v*/*v*)			
	Col-69	**PV-1457**	**LA2**	**LI3**
Fennel	2.51 ± 0.18 b/B	7.79 ± 0.03 a/A	7.49 ± 0.06 a/A	1.04 ± 0.012 d/C
Laurel	6.65 ± 0.58 a/D	2.29 ± 0.17 b/B	6.3 ± 0.04 c/C	2.1 ± 0.009 b/A
Peppermint	2.82 ± 0.01 b/D	7.43 ± 0.02 a/A	6.83 ± 0.16 b/B	3.27 ± 0.05 a/C
Citronella	1.81 ± 0.002 c/AB	1.1 ± 0.18 c/C	2.3 ± 0.17 d/A	1.63 ± 0.3 c/BC
Lavender	2.6 ± 0.06 b/A	1.75 ± 0.35 b/B	1.96 ± 0.02 e/B	0.40 ± 0.003 e/C
Clove	0.53 ± 0.09 d/D	1.06 ± 0.001 c/B	0.83 ± 0.05 f/C	1.98 ± 0.01 b/A
Garlic	0.99 ± 0.01 d/A	0.45 ± 0.001 d/C	0.75 ± 0.04 f/B	0.42 ± 0.005 e/C
p-value	≤0.0001	≤0.0001	≤0.0001	≤0.0001

Data present the mean of three replicates from two replicated experiments ± standard error; Means followed by common lowercase letters in a column, or by uppercase letters in a row, do not differ significantly according to Fisher's protected LSD test ($p = 0.05$).

Table A5. EC_{50} and EC_{90} values (% *v*/*v*) of nanoemulsified essential oils (N-EOs) inhibiting the conidial germination of *Neofusicoccum parvum* isolates (AGR-CT-27 and AGR-CT-84).

N-EOs	EC_{50} Values (% *v*/*v*)	
	AGR-CT-27	**AGR-CT-84**
Fennel	2.55 ± 0.11 b/A	2.8 ± 0.04 b/A
Laurel	3.25 ± 0.05 a/B	5.17 ± 0.16 a/A
Peppermint	2.39 ± 0.003 b/A	2.32 ± 0.03 c/A
Citronella	2.55 ± 0.06 b/B	2.89 ± 0.03 b/A
Lavender	1.20 ± 0.18 c/A	0.99 ± 0.02 d/A
Clove	0.43 ± 0.08 d/A	0.37 ± 0.003 e/A
Garlic	<0.1 [x] e/A	<0.1 [x] f/A
p-value	≤0.0001	≤0.0001
	EC_{90} values (% *v*/*v*)	
	AGR-CT-27	**AGR-CT-84**
Fennel	2.96 ± 0.15 c/A	3.51 ± 0.31 c/A
Laurel	5.44 ± 0.11 a/B	8.09 ± 0.02 a/A
Peppermint	3.73 ± 0.06 b/A	3.81 ± 0.06 c/A
Citronella	5.69 ± 0.16 a/A	5.8 ± 0.10 b/A
Lavender	1.38 ± 0.28 d/A	1.08 ± 0.03 d/A
Clove	0.49 ± 0.07 e/A	0.42 ± 0.0 e/A
Garlic	<0.1 [x] f/A	<0.1 [x] f/A
p-value	≤0.0001	≤0.0001

Data present the mean of three replicates from two replicated experiments ± standard error; Means followed by common lowercase letters in a column, or by uppercase letters in a row, do not differ significantly according to Fisher's protected LSD test ($p = 0.05$). [x] Total inhibition at the lower concentration tested (0.1% *v*/*v*).

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