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Potential of essential oils in inhibiting the phytopathogenic bacteria *Ralstonia solanacearum* and *Pectobacterium carotovorum*

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Abstract

Brazilian agricultural production is often threatened by phytopathogenic bacteria, resulting in significant economic losses. Difficult to control and favored by the country's climatic conditions, bacterial wilt, caused by *Ralstonia solanacearum* and soft rot, by *Pectobacterium carotovorum,* are major diseases that affect a wide range of hosts. As an alternative control strategy, this study was developed to evaluate the chemical composition and antimicrobial activity of essential oils from *Elionurus latiflorus* (Brazilian lemongrass) and *Citrus aurantium* (bitter orange). The main components identified were citral (65.38 %) in *E. latiflorus* and limonene (94.71 %) in *C. aurantium*. The antimicrobial activity of the essential oils was assessed through disk diffusion assays, using concentrations of 1, 3, 5, 7, 10, and 15 μL for *E. latiflorus* oil and 1, 3, 5, 7, 10, 15, 20, and 30 μL for *C. aurantium* oil. *E. latiflorus* oil had strong antimicrobial effects against both bacteria. At 15 μL, inhibition rates reached 86.46 % for *R. solanacearum* and 88.90 % for *P. carotovorum*. In contrast, *C. aurantium* oil had no inhibitory activity against the tested bacteria. These findings suggest that *E. latiflorus* essential oil may represent a natural, ecological, and efficient alternative for management of phytopathogenic bacteria.

Keywords: Alternative control. Soft rot. Bacterial wilt.

Introduction

With the growing global demand for food and significantly stagnated agricultural production, feeding the population adequately has become an even greater challenge. In order to maximize their potential, producing countries need to manage major agricultural threats such as climate change and diseases caused by microorganisms. It is estimated that 10 to 28 % of global food production is lost due to diseases caused by bacteria, fungi, and virus, resulting in an estimated loss of 220 billion dollars (Hemanthilake, Gunathilake, 2022; Silva, 2022).

As a major contributor to global food production and supply, Brazil has been considered a strategic source of food for humanity. Its tropical climate favors the cultivation of various crops but also creates ideal conditions for the development of numerous pathogens, constantly threatening both the productivity and quality of food (Vieira Junior *et al*., 2019; Batista *et al*., 2020).

With over 7,000 cataloged species, at least 150 of which are considered phytopathogenic, bacteria affect agricultural crops worldwide. Capable of colonizing and multiplying within plant tissues, they cause diseases such as wilt, rot, chlorosis, nutrient loss, and growth disorders. Key associated genera include *Agrobacterium*, *Pseudomonas*, *Xanthomonas*, *Pectobacterium*, and *Ralstonia* (Gasparotto, Pereira, Schurt, 2024; Gotti, 2018).

Affecting more than 50 different crops, *Ralstonia solanacearum* causes bacterial wilt. This soil-borne bacterium inhabits the plant's vascular system, multiplies, and blocks water transport in the xylem, leading to wilting of leaves and stems and ultimately plant death. With over 650 strains isolated in Brazil alone, this disease poses an increasing threat to many other hosts, especially in tropical and subtropical regions (Lopes, Rossato, 2018).

Bacteria from the genus *Erwinia*, known for their ability to produce pectinolytic enzymes, destroy the parenchymal tissues of plants, causing a disease known as "soft rot." This results in the discoloration of tubers and fruits, rapid softening, and decay. With a strong capacity for movement in the soil, species of this genus are considered among the most destructive (Carvalho Filho, Mello, 2018; Jeyakumar *et al*., 2024).

These bacteria are typically managed with bactericides—broad-spectrum synthetic agrochemicals—designed to eliminate pathogens. However, the once-optimistic view that pesticides could solve all agricultural problems, including food quantity and quality issues, is no longer widely accepted. The extensive use of these chemicals has been increasingly linked to environmental issues such as soil and water contamination, health problems due to acute and chronic intoxication, and the emergence of super-resistant bacterial strains (Pereira, Borges, 2020; Matias et al., 2021).

Currently, there is a growing effort to identify natural products with antimicrobial properties that may effectively control pathogens without compromising food safety. Unlike traditional chemical agents, these natural products are less harmful to human health and the environment. In this context, essential oils, volatile aromatic substances with strong biological activity (bactericidal, fungicidal, and insecticidal), have emerged as a promising alternative (Li, Qiao, Zhang, 2025; Souza *et al*., 2025; Ivanova *et al*., 2025).

Extracted from various parts of plants, these oils contain a complex mixture of alcohols, phenols, esters, and terpenes that act in a synergistic and unique manner. Their biological activity involves mechanisms such as repellency, growth inhibition, and toxin production suppression. In food systems, significant effects have been observed when these compounds are applied both pre- and post-harvest (Fazolin *et al*., 2023).

Many traditional plant species are being studied as potential alternatives to the use of pesticides in agriculture. Citrus plants such as *Citrus aurantium*, commonly known as bitter orange, and native flora species like *Elionurus latiflorus*, known as Brazilian lemongrass, are examples of plants that have good adaptability to climate conditions. Their essential oil compositions feature a wide range of valuable compounds, including linalool, limonene, neral, geranial, citronellal, and myrcene (Brahmi *et al*., 2021; Oulebsir et al., 2022; Ahranjani, Esfandiari, Nodeh, 2025).

Given the above, the present study was developed to evaluate the inhibitory potential of the essential oils from *Elionurus latiflorus* and *Citrus aurantium* on the growth of the bacteria *Ralstonia solanacearum* and *Pectobacterium carotovorum*.

Materials and methods

Microorganisms: cultures and growth medium

Cultures of the bacteria *Ralstonia solanacearum* and *Pectobacterium carotovorum* were used, maintained at the Biotechnology and Post-Harvest Laboratory of the Escola de Engenharia de Volta Redonda (EEIMVR/UFF).

The microorganisms were grown in nutrient broth composed of meat extract (0.45 g), casein peptone (0.75 g), and distilled water (150 mL).

Analysis of essential oil constituents

The essential oils of bitter orange (*Citrus aurantium*) and Brazilian lemongrass (*Elionurus latiflorus*) were obtained from a company, which reported limonene and citral as their main constituents, respectively. For a more detailed characterization of the chemical composition of these oils, an analysis was performed using gas chromatography coupled with mass spectrometry (GC/MS). A capillary column made of fused silica with a DB-5 stationary phase was used (0.25 μm film thickness, 30 mm length, and 0.25 mm internal diameter). Helium gas was used as the carrier at a flow rate of 1.0 mL min^{-1}. The injector temperature was set at 220°C, and the detector at 240°C. The initial oven temperature was maintained at 60°C for two minutes, then programmed to increase by 3°C per minute until reaching a maximum temperature of 240°C, which was held for 30 minutes, resulting in a total run time of 91 minutes.

A 1:20 split ratio was used, with a solvent cut-off time of 5 minutes. The sample injection volume was 1 μL at a concentration of 10,000 ppm, using hexane as the solvent. The identification of the compounds was performed by comparing the obtained mass spectra with those in the equipment's database and by using the Kovats Retention Index (KI) of each component. The quantitative analysis of the main components of the essential oil, expressed as a percentage, was carried out using the peak area normalization method, as described by Zhang *et al*. (2006).

Evaluation of bactericidal activity

The antimicrobial activity of the essential oils was assessed using a modified agar diffusion technique with sterile filter paper discs (Bauer *et al*., 1966). According to Falcão *et al*. (2017), this method can be used as a preliminary evaluation technique, as it is recognized for determining the sensitivity of various microorganisms to specific essential oils.

Discs with a diameter of 10 mm were impregnated with different concentrations of each tested oil: 1, 3, 5, 7, 10, and 15 μL mL^{-1} for *E. latiflorus* oil, and 1, 3, 5, 7, 10, 15, 20, and 30 μL mL^{-1} for *C. aurantium* oil, diluted in the surfactant agent DMSO (dimethyl sulfoxide) at 1 % v/v. These discs were placed on 90 mm Petri dishes containing Mueller-Hinton agar inoculated with bacterial strains suspended at a concentration of 1.5×10^8 CFU mL^{-1}. The plates were incubated at 37 ± 1°C for 24 hours, after which the inhibition zones were measured in millimeters. The procedure was performed in triplicate. Sterile discs with no compounds were used as negative controls. In the negative control treatment, all bacteria reached the maximum diameter of 90 mm, indicating the fertility of the culture medium.

Statistical analysis

The experiment was conducted in a completely randomized design with seven treatments and five replicates. The data presented in the tables and graphs are expressed as mean ± standard deviation. Statistical analysis was performed using the SISVAR® software, with analysis of variance (ANOVA) and mean comparisons using Tukey's test at a 5 % significance level.

Results and discussion

Composition of essential oils

The components of the essential oil of Brazilian lemongrass (*Elionurus latiflorus*) are presented in Table 1, identified through chromatographic analysis.

Table 1. Main components of the essential oil of Brazilian lemongrass (*Elionurus latiflorus*) determined by GC-MS.

COMPONENTS	RETENTION TIME (min)	AREA (%)
Beta-myrcene	5,412	3,42
Linalool	9,499	4,26
Carane	13,091	2,87
Neral	15,888	28,72
Geraniol	16,367	3,49
Lavandulol	16,598	3,46
Geranial	17,363	36,66
Geranyl acetate	21,990	8,45
Elixene	26,072	8,65

Source: authors (2025).

The analysis of the essential oil constituents of *E. latiflorus* (Table 1) identified the presence of nine components, including monoterpene alcohols and terpenes. Among these, two components stood out due to their higher concentrations: neral (28.72 %) and geranial (36.66 %), both constituents of citral, together accounting for 65.38 % of the essential oil. Similar data were reported by Faria *et al*. (2023), in which geranial and neral made up the majority (91.5 %) of the 15 compounds identified in *E. latiflorus* oil.

These oxygenated monoterpenes, such as citral, are of great interest due to their antimicrobial properties, which are applicable to diseases affecting humans, animals, and plants. Citral can penetrate the microbial cell wall, acting on protein denaturation and consequently causing the destruction of the cell membrane, leading to cell death (Saddiq, Khayyat, 2010). Antibacterial, antiparasitic, and antifungal activities have already been reported in the literature (Gehan *et al*., 2018; Atolani *et al*., 2019; Long *et al*., 2019; Martinazzo, Braga, Teodoro, 2022).

The components of the essential oil of bitter orange (*Citrus aurantium*) were described in Table 2.

The essential oil of *Citrus aurantium* was predominantly composed of limonene, which accounted for 94.71 % of its composition. High limonene levels are a common feature of citrus fruits, typically ranging from 92.52 to 97.3 %; for instance, grapefruit contains 93.33 % and tangerine 95.95 % (Bier *et al*., 2011; Singh *et al*., 2021). Limonene is a major terpene widely distributed in nature and represents a key constituent of numerous essential oils. However, the composition of essential oils can vary considerably depending on environmental conditions, plant organs, harvest time, genetic background, and extraction methods

Table 2. Main components of the essential oil of bitter orange (*Citrus aurantium*) determined by GC-MS.

COMPONENTS	RETENTION TIME (min)	AREA (%)
Not identified	7,123	-
Myrcene	9,085	-
Limonene	10,77	94,71
Linalool	13,648	-

Source: authors (2025).

(Seyyedi-Mansour *et al*., 2025). In addition, compounds such as linalool and linalyl acetate are often reported in other plant parts, including flowers and leaves (Elhawary *et al*., 2024).

It is important to emphasize that the extraction method directly influences the composition of the essential oil. Using the hydrodistillation technique, Brahmi et al. (2021) obtained 93.38 % limonene from the peel of bitter orange, a result quite similar to that found in the present study. However, when microwave-assisted hydrodistillation was applied, this percentage decreased to 59.96 %.

Among the main activities of these compounds, limonene exhibits antifungal (Yu et al., 2022), larvicidal (Campolo *et al*., 2016) and insecticidal (Isman, 2020) properties. Regarding linalool, studies have confirmed its use in inhibiting fungal and bacterial growth, showing better results against Gram-positive bacteria by acting on protein denaturation or dehydration of vegetative cells (Soković *et al*., 2010; Yang, Chao, Liu, 2014; Fajdek-Bieda *et al*., 2024). Although myrcene does not have proven antimicrobial effects, when combined with compounds that do, such as citral, it can either enhance their effect or act antagonistically (Bakkali *et al*., 2008; Silva *et al.*, 2013).

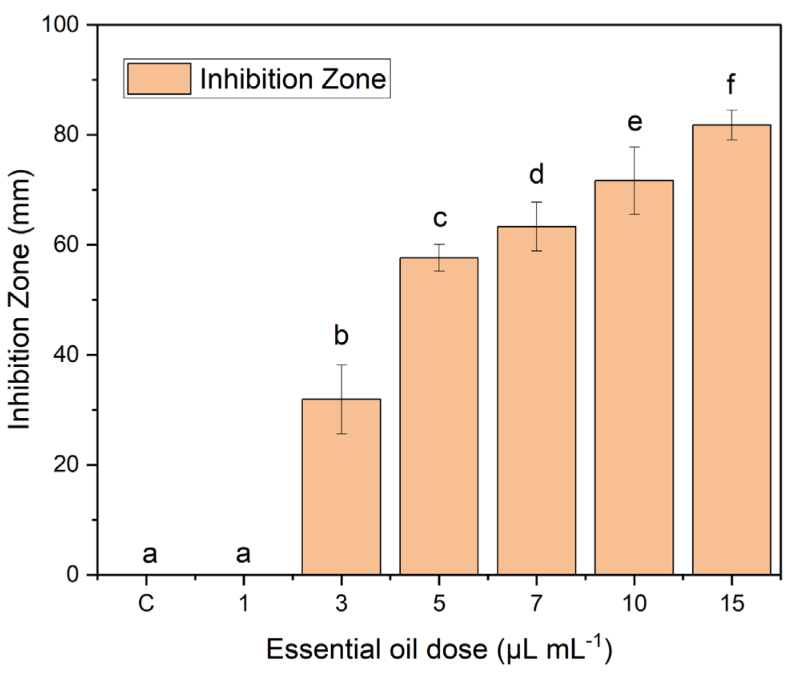
Effect of antimicrobial activity

Essential ail: *Elionurus latiflorus*

The percentages of growth inhibition of the bacterium *Ralstonia solanacearum* under different treatments (0, 1, 3, 5, 7, 10, and 15 µL mL^{-1}) with essential oil of *Elionurus latiflorus* are presented in Figure 1.

From Figure 1, it is evident that the concentration influenced the inhibition of bacterial growth. The highest dose tested, 15 µL, produced the largest inhibition zone of 79.56 mm, which corresponds to 88.4 % of the

Figure 1. Effect of *Elionurus latiflorus* essential oil on the growth of the phytopathogenic bacterium *Ralstonia solanacearum.* C = Control (0 µL). Columns with different letters indicate statistically significant differences according to Tukey's test ($p < 0.05$).



Source: authors (2025).

total plate area, significantly differing from the other doses tested. A proportional relationship can also be observed, where the higher the dose of essential oil applied, the larger the inhibition zone achieved.

Similar results were found by Martinazzo, Braga and Teodoro (2022), where the essential oil of *E. latiflorus* promoted an average inhibition zone of 85.81 mm at the 15 µL dosage against *R. solanacearum*. For higher concentrations (20, 30, and 40 µL), the inhibition percentage did not differ significantly, with values around 90 mm.

Abd-Elrahim *et al*. (2022) demonstrated the effects of various essential oils. Anise (*Pimpinella anisum*), thyme (*Thymus vulgaris*), clove (*S. aromaticum*), fennel (*Foeniculum vulgare*), and lemongrass (*C. flexuosus*), at concentrations of 8, 14, and 28 µL, were tested for treating bacterial wilt caused by *R. solanacearum*, achieving good results at all concentrations with inhibition zones ranging from 20.3 to 66.7 mm.

The efficiency of *E. latiflorus* essential oil can be compared to that of other oils. Ashmawy *et al*. (2020) evaluated the effect of pine oil (*Pinus halepensis*) on *R. solanacearum*, reporting an inhibition zone of 15.33 mm, which is lower than that obtained with one of the smallest doses in this study, 3 µL, which had an inhibition zone of 31.62 mm. For rosemary (*Rosmarinus officinalis*) and lemon balm (*Lippia alba*) essential oils, Martins *et al*. (2011) reported inhibition zones equal to or less than 40 mm with doses between 0.25 and 8 µL, similar to the 5 µL dosage of *E. latiflorus* found in this study. These data indicate that even at lower doses, *E. latiflorus* essential oil has satisfactory bactericidal results.

Studies where the major constituent found in the essential oil was citral, as in the oil of *E. latiflorus*, demonstrated good results in inhibiting bacterial diseases. Amorim *et al*. (2011) observed the bactericidal potential of citronella oil (*Cymbopogon nardus*) in controlling *R. solanacearum*, with total inhibition of banana moko disease. Lee et al. (2012) demonstrated the antibacterial effect of cinnamon oil (*Cinnamomum verum*) against *R. solanacearum*, with an inhibition zone of 56.60 mm at 10 µL of the oil.

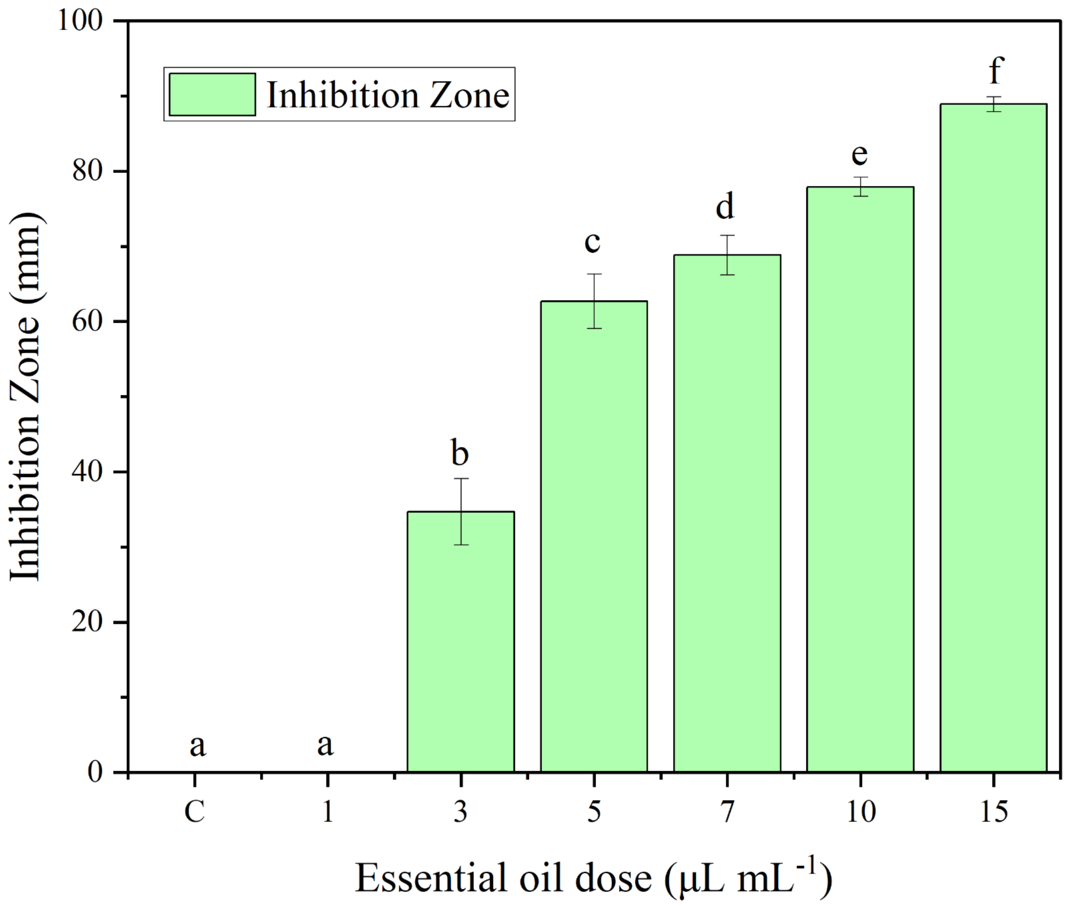
The percentages of growth inhibition of the bacterium *P. carotovorum* under different treatments (0, 1, 3, 5, 7, 10, and 15 µL mL^{-1}) with *E. latiflorus* essential oil can be seen in Figure 2.

From Figure 2, a behavior similar to that observed with the bacterium *R. solanacearum* is noted, where the 15 µL dose reached the highest performance in inhibiting *P. carotovorum* with an inhibition halo of 81.79 mm, representing 90.87 % of the plate area, compared to the other doses, which also differed significantly from each other. It is also observed that as the oil concentration increases, there is an apparent increase in the inhibition halo.

Considering the main bioactive agent, citral, Marei, Rabea e Badawy (2018) demonstrated the compound's ability to inhibit *P. carotovorum* and other microorganisms, being recognized as an economical and effective way to protect crops against microbial pathogens. Almeida *et al*. (2020) achieved total inhibition in controlling this pathogen with 25 µL doses of *Cymbopogon citratus* essential oil. Other essential oils, such as caraway (*Carum carvi*), cinnamon (*Cinnamomum zeylanicum*), cumin (*Cuminum cyminum*), clove (*Eugenia caryphyllus*), fennel (*Foeniculum vulgare*), mint (*Mentha piperita*), oregano (*Origanum vulgare*), tea tree (*Malaleuca alternifolia*), rosemary (*Rosmarinus officinalis*) and thyme (*Thymus vulgaris*), proved effective in inhibiting *P. carotovorum* (Jílková *et al*., 2025).

The importance of synergism between compounds can be seen in the work of Zhang

Figure 2. Effect of *Elionurus latiflorus* essential oil on the growth of the phytopathogenic bacterium *Pectobacterium carotovorum*. C = Control (0 μL). Columns with different letters indicate statistically significant differences according to Tukey's test ($p < 0.05$).



Source: authors (2025).

et al. (2021). While citral had low inhibitory effect on biofilms of mixed species including the genus *Erwinia*, geraniol promoted a 61.27 % inhibition, acting on quorum sensing and the secretion of exopolysaccharides.

It is worth noting that the potential of this oil goes beyond its antimicrobial activity. Faria *et al*. (2023) demonstrated that the oil extracted from *E. latiflorus* leaves, both individually and in binary mixtures, was able to significantly reduce the production of aflatoxins B1, B2, G1, and G2 in fungi associated with the Mexican bean weevil (*Zabotes subfasciatus*), an effect mainly attributed to its high citral content.

Essential oil: *Citrus aurantium*

The analysis of the antimicrobial activity of *C. aurantium* essential oil against the bacteria *R. solanacearum* and *P. carotovorum* showed no inhibition at any of the tested doses.

A similar result was reported by Li *et al*. (2019) using *Citrus medica*, also rich in limonene (45.36 %), where low control of gram-negative bacteria was observed compared to gram-positive bacteria. Shakeri *et al*. (2014) and Thinh *et al*. (2022) explain the lower effectiveness of essential oils or their compounds, such as limonene, on gram-negative bacteria due to the presence of the outer membrane rich in lipopolysaccharides, which provides a barrier to compound penetration, effectively filtering it. For example, Silva *et al*. (2021) demonstrated a low effect of limonene on species such as *Aeromonas hydrophila*, *Citrobacter freundii*, *Raoultella ornithinolytica*, and *Stenotrophomonas maltophilia*. This behavior has been frequently reported in the literature (Zomorodian *et al*., 2018; Ghavam *et al*., 2020; Huong *et al*., 2024).

In a study with gram-positive bacteria of the genus *Staphylococcus aureus*, Han, Chen, and Sun (2021) confirmed the bactericidal effect through cell membrane destruction and increased permeability at a concentration of 20 mL of limonene. Van Vuuren and Viljoen (2007) emphasized that the antibacterial effect of limonene increased when combined with other compounds in an essential oil, acting synergistically and enhancing its biological activity rather than acting alone.

The type of enantiomer of this monoterpene present in the essential oil also seems to influence antibacterial activity. Lee et al. (2016) reported good inhibitory performance of the S-limonene compound against gram-negative bacteria of the species *Xanthomonas oryzae* pv. *Oryzae*, phytopathogens in foods such as rice. Furthermore, it was observed that higher concentrations of the compound (3 to 5 mM) led to greater inhibition percentages, which may indicate that the antibacterial activities of these compounds may relate not only to pathogen specificity but also to the enantiomeric form.

Among the alternatives evaluated for the use of essential oils as antimicrobial agents in controlling bacterial growth, the essential oil of *Elionurus latiflorus* stood out for its high efficacy against the phytopathogenic bacteria *Ralstonia solanacearum* and *Pectobacterium carotovorum*. It is believed that concentrations higher than those tested could further enhance this antimicrobial activity, potentially leading to complete inhibition of the phytopathogens.

Conclusions

The major constituents of the essential oils were citral (65.38 %) in Brazilian lemongrass (*Elionurus latiflorus*) and limonene (94.71 %) in bitter orange (*Citrus aurantium*). The antimicrobial activity of these oils was shown to depend on both their chemical composition and applied concentration. While *C. aurantium* oil, rich in limonene, exhibited no inhibitory effect against the tested bacteria, *E. latiflorus* oil, rich in citral, demonstrated significant efficacy at the highest concentration tested (15 μL), reaching inhibition rates of 88.4 % for *Ralstonia solanacearum* and 90.87 % for *Pectobacterium carotovorum*. These results highlight the potential of *E. latiflorus* essential oil, particularly its citral content, as a natural and sustainable antimicrobial agent for the control of phytopathogenic bacteria in agriculture.

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