

Chemistry

Alternative control of phytopathogenic bacteria with essential oils of *Elionurus latiflorus* and *Cymbopogon flexuosus*

Controle alternativo de bactérias fitopatogênicas com os óleos essenciais de *Elionurus latiflorus* e *Cymbopogon flexuosus*

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ABSTRACT

The use of hazardous chemicals has become a common practice to control diseases that affect agricultural production. In this scenario, society is challenged to increase food availability while reducing pesticide use, which causes damage to health and the environment. In this sense, essential oils appear as a promising alternative to reduce the use of pesticides, since they are plant-derived compounds. This study aimed to identify the main chemical components and evaluate the in vitro antimicrobial potential of essential oils of the Brazilian species *Elionurus latiflorus* and the exotic species *Cymbopogon flexuosus* against the phytopathogenic bacteria *Xanthomonas axonopodis* pv. *phaseoli*, *Ralstonia solanacearum*, *Pectobacterium carotovorum* pv. *carotovorum* and *Pseudomonas syringae* pv. *tomato*. The main component identified of the essential oils was citral at the concentrations 65.38% for *E. latiflorus* and 71.6% for *C. citratus*. The analysis of the antibacterial activity of the essential oils showed effect against all bacteria analyzed when compared to the antibiotic gentamicin. The antibiotic produced inhibition zone diameters from 11.30 to 20.67 mm, while the essential oils produced the inhibition zones from 51.22 to 90 mm and pure citral around 86 mm. MIC values for essential oils were found between 25 and 200 $\mu\text{L}/\text{mL}$ and MBC between 100 and 400 $\mu\text{L}/\text{mL}$. The study showed that the oils have inhibitory effect on the microorganisms tested.

Keywords: Antimicrobial; MIC; MBC; Citral

RESUMO

A utilização de produtos nocivos tornou-se prática corriqueira no controle de doenças que afetam a produção agrícola. Diante deste cenário a sociedade se depara com o desafio de aumentar a disponibilidade de alimentos e diminuir a aplicação de agrotóxicos, os quais causam danos à saúde e ao meio ambiente. Neste sentido, os óleos essenciais surgem como alternativa promissora para reduzir o uso de defensivos agrícolas, por se tratar de compostos de origem vegetal. O objetivo deste estudo foi identificar os principais componentes químicos e avaliar o potencial antimicrobiano in vitro dos óleos essenciais da espécie brasileira *Elionurus latiflorus* e da exótica *Cymbopogon flexuosus* e no controle das bactérias fitopatogênicas *Xanthomonas axonopodis* pv. *phaseoli*, *Ralstonia solanacearum*, *Pectobacterium carotovorum* pv. *carotovorum* e *Pseudomonas syringae* pv. *tomato*. O principal componente dos óleos essenciais identificado foi o citral nas concentrações de 65,38 e 71,6% para *E. latiflorus* e *C. citratus*, respectivamente. A análise da atividade antibacteriana dos óleos essenciais demonstrou atividade contra todas as bactérias analisadas quando comparados ao antibiótico gentamicina que apresentou halos de inibição variando de 11,30 a 20,67 mm, enquanto para os óleos testados os halos de inibição variaram de 51,22 a 90 mm e para o citral puro de 86 mm em média. Foram encontrados valores para a CIM entre 25 a 200 µL/mL e para CBM de 100 a 400 µL/mL para os óleos essenciais. Foi possível verificar que os óleos possuem efeito inibitório sobre os microrganismos estudados.

Palavras-chave: Antimicrobiano; CIM; CBM; Citral

1 INTRODUCTION

Over the years, world agricultural production has been affected by diseases caused by bacteria, fungi, and insects, which reduce approximately 10% to 16% of the food supply. The yield of wheat, rice, and soybeans have dropped by 21.5%, 30%, and 21.4%, respectively, as well as interfering with the quantity and quality of food (WAHABZADA *et al.*, 2015; SILVA *et al.*, 2018; CARVAJAL-YEPES *et al.* 2020).

An additional persistent concern is that productivity gains and control of phytopathogenic bacteria are linked to the pesticide use, while its indiscriminate use is associated with increased environmental damage and risks to human health. (CARNEIRO *et al.*, 2015; SOUZA *et al.*, 2017; SIDDIQUE *et al.*, 2017; REYNOSO *et al.*, 2019).

There is, therefore, a growing demand for natural substances as alternative to pesticide use, aiming to control agricultural pathogens. Compounds of plant origin provide a new strategy to pest management due to their low toxicity to mammals, low environmental persistence, minimal residual activity and, therefore, wide public acceptance (KAISER *et al.*, 2016; SILVA *et al.*, 2018; BENALI *et al.*, 2020).

Phytopathogenic bacteria account for the main losses in crops such as citrus, cassava, banana, rice, wheat, sugarcane, and beans. Their mechanism of action cause symptoms including spots, cankers, rots, and hormonal imbalances that lead to excessive plant growth, dwarfism, branching of roots, and epinastic leaf growth, among others (NADARASAH and STAVRINDES, 2011; TIAN *et al.*, 2016; MARTINS *et al.*, 2018; ABDULAI *et al.*, 2018).

Given the diversity of species that produce essential oils and the variety of their chemical composition, studies show that among these oils, those presenting the citral constituent are promising in the control of microorganisms that affect plants. *Elionurus latiflorus* is native to a large part of the Brazilian coast and popularly known as “capim-barba-de-bode” or Brazilian lemon grass. It is among the Brazilian species that contain citral as the major constituent but has been scarcely studied to date. An exotic species, but easy to grow in Brazil and also presenting the citral as major component is *Cymbopogon flexuosus*, from East India and commercially named lemongrass, which is used in various cosmetic and pharmaceutical industries (MURIEL-GALET *et al.*, 2012; GONÇALVES *et al.*, 2013; FÜLLER *et al.*, 2014; ALKAN and YEMENICIOGLU, 2016; ADHIKARI *et al.*, 2017).

Therefore, the objectives of the present study are to identify the chemical composition and evaluate the antibacterial properties of the essential oils of *Elionurus latiflorus* and *Cymbopogon flexuosus* in the control of the phytopathogenic bacteria *Xanthomonas axonopodis* pv. *phaseoli*, *Ralstonia solanacearum*, *Pectobacterium carotovorum* pv. *carotovorum*, and *Pseudomonas syringae* pv. *tomato*.

2 MATERIAL AND METHODS

The essential oils and the antibiotic used in the experiment were purchased from companies in the sector.

The analysis of essential oil constituents was performed by gas chromatography-mass spectrometry (GC/MS). A fused-silica capillary column with DB-

5 stationary phase 0.25µm thick, 30m long and 0.25mm internal diameter) was used for separations. Helium was used as carrier gas at a flow rate of 1.0mL/minute. The temperature of the injector was hold at 220°C and the detector at 240°C. The initial oven temperature was maintained at 60°C for 2min and programmed with a heating rate of 3°C min⁻¹ to 240°C and held for 30min, in a total analysis time of 91 minutes. The split ratio was 1:20 and the solvent cut-off time was 5 minutes. The sample injection volume was 1µL, at a concentration of 10,000ppm, using hexane as solvent. Compounds were identified by comparing the mass spectra obtained with those of the apparatus database and by the Kovats Retention Index (IK) of each component (LANÇAS, 1993). The quantitative analysis of the main components of the essential oil, expressed as percentage, was performed by the peak area integration normalization method, as described by (ZHANG *et al.*, 2006).

The evaluation of the antimicrobial activity of essential oils was performed using three techniques: agar diffusion, Minimum Inhibitory Concentration, and Minimum Bactericidal Concentration.

The agar diffusion or plate diffusion test was used as a prior evaluation method, as it is recognized for the determination of the sensitivity of many microorganisms to certain essential oils, easiness to perform, and demand of small amounts of samples (ROMAN *et al.*, 2017). In this technique, sterile filter paper discs of 10mm in diameter were soaked with the essential oils and placed on 90mm Petri dishes containing Mueller Hinton agar inoculated with the strains of all bacteria in suspension in the concentration of 1.5x10⁸ CFU/mL. Sterile paper discs were used as negative control and the antibiotic gentamicin (20µg/mL) as positive control. The plates were incubated at 37 ± 1 °C for 24 hours, then the inhibition zones were measured in millimeters (BAUER *et al.*, 1966), with five repetitions.

The antibiotic concentration was tested with the doses 20, 30, 50, 100, and 200µg/mL for *Pseudomonas* and *Xanthomonas*, as these bacteria were found more resistant in the pre-tests. Afterwards, the statistical analysis showed no significant differences between the doses tested, thus the lowest dose of 20µg/mL in the

preliminary tests was chosen. At the end of the experiment, a preliminary test was carried out to indicate the antibacterial activity of pure Citral at doses that showed a similar inhibition zone with both essential oils tested.

To determine the lowest concentration of antimicrobial capable of inhibiting the microorganism (MIC – Minimum Inhibitory Concentration), the agar well diffusion method adapted from Bauer *et al.* (1966). A serial dilution of the essential oil was prepared, in which 100 µL of culture medium and 5% Dimethylsulfoxide (DMSO) were added 800µL of essential oil diluted in 100µL of culture medium and 5% DMSO. A 100µL aliquot of this solution was homogenized with 100µL of medium and 5% DMSO in the next well, and so on, obtaining a range of essential oil concentrations (360µg/mL in the first well and 2.8125µg/ml in the last well). A 10µL aliquot of bacterial solution was added to each well. As controls, wells with culture media were used with the bacterial solution and without the bacterial solution. Afterwards, the microplates were incubated at $37 \pm 1^\circ\text{C}$ for 24 hours. MIC was determined by reading the plates, using an automatic microplate reader with a pre-selected wavelength of 490nm, to confirm the presence of bacterial growth in the culture medium. The results were calculated by subtracting the readings of the essential oil from the reading of the control treatment. All procedures were aseptic and conducted in a laminar cabinet (FARIAS *et al.*, 2019).

The Minimum Bactericidal Concentration (MBC) was determined by the macro-dilution method carried out in tubes, based on the Clinical & Laboratory Standards Institute (CLSI) (PATEL *et al.*, 2015). The oil concentrations used in the experiments were based on the results of the MIC tests. The inoculum suspension of each bacteria was added to each sterilized tube containing Muller Hinton broth with different final concentrations of essential oil solubilized with 5% DMSO. The tubes were incubated at $37 \pm 1^\circ\text{C}$ for 24 hours. Controls containing culture medium without the microorganism and with the microorganism were carried out. The tests were performed in triplicate. Following, 100µL aliquots from each tube were transferred to Petri dishes containing the agar medium and incubated at 37°C for 24 hours. Determination of MBC of essential oil was performed by eye, based on the fact that

these bacteria grow on the plates forming cell clusters or the so-called growth knobs. Thus, the lowest concentration of essential oil that totally inhibits visible bacterial growth indicates MBC.

The experiment was arranged in a completely randomized design. The results were analyzed by analysis of variance and means compared by the Scott-Knott test at 5% significance level.

3 RESULTS AND DISCUSSION

3.1 Essential oils components

Table 1 shows the average retention time and percentage peak area of the components identified in the essential oils of *Elionurus latiflorus* and *Cymbopogon flexuosus* by chromatography.

Table 1 - Main components of essential oils of *Elionurus latiflorus* and *Cymbopogon flexuosus* identified by GC-MS

Component	<i>Elionurus latiflorus</i>		<i>Cymbopogon flexuosus</i>	
	Retention time (min)	Area (%)	Retention time (min)	Area (%)
6-Methyl-5-hepten-2-one	-	-	8.927	1.88
Geranyl Acetate	21.990	8.45	26.664	6.16
Beta myrcene	5.412	3.42	-	-
Carane	13.091	2.87	-	-
Caryophyllene	-	-	28.315	2.85
Citronella	-	-	16.097	0.48
Elixene	26.072	8.65	-	-
Geranial	17.363	36.66	21.768	39.79
Geraniol	16.367	3.49	20.873	9.45
Lavandulol	16.598	3.46	-	-
Linalool	9.499	4.26	13.647	1.35
Neral	15.888	28.72	20.353	31.81

Source: Authors' (2021)

The main component identified in the essential oils was citral at the concentrations of 65.38% for *E. latiflorus* and 71.6% for *C. citratus*. Citral is a mixture of the isomers geranial, at concentrations of 36.66% and 39.79%, and neral, at concentrations of 28.72% and 31.81%.

The results for the genus *Cymbopogon* were similar to those reported by Pandey *et al.* (2003), Tajidin *et al.* (2012), Lermen *et al.* (2015), and Feriotto *et al.* (2018). The authors evaluated the chemical composition of the *C. citratus* essential oil and found concentrations of geranial varying from 20%-50% and neral from 30% - 40%.

Conversely, no studies were found on the composition of *Elionurus latiflorus* essential, but Füller *et al.* (2014) evaluated the composition of *Elionurus muticus* and found concentrations of 31.54% of neral and 47.33% of geranial, which agree with the results obtained in this work.

It is to be noted that citral has been identified as the main constituent of other plants, including *Cymbopogon citratus* (KUMAR *et al.*, 2013), *Elionurus muticus* (FÜLLER *et al.*, 2014), and *Lippia alba* (FARIAS *et al.*, 2019). Furthermore, studies have proven that this component possess antibacterial, antitumor, and antiparasitic effects (ALKAN & YEMENICIOĞLU, 2016; LONG *et al.*, 2019; GAO *et al.*, 2020).

In addition to citral, other compounds were identified in both essential oils such as linalool (1.35 and 4.26%), geraniol (9.45 and 3.49%), and geranyl acetate (6.16 and 8.45%). The antibacterial activity of linalool (ASMAA & NADJIB, 2017) and geraniol (BALTA *et al.*, 2017) has also been demonstrated.

The monoterpene citral, as major component, has antifungal activity against pathogens that affect humans and plants, inhibits seed germination, and has bactericidal and insecticidal properties. According to Rossi *et al.* (2017), the composition of the essential oil significantly interferes in the study of bacterial control, since its antibacterial activity is not ascribed to a specific mechanism, that is, the chemical structure of the components affects its precise mode of action and antibacterial activity.

Rahman and Kang (2009) state that the risk that pathogenic microorganisms develop resistance to essential oils is very low due to the composition of several antimicrobial substances, which act through different mechanisms. This is an advantageous characteristic of essential oils over other antimicrobial agents and can bring benefits to the various areas that can be applied.

3.2 Antibiotic antibacterial activity

The antibiotic gentamicin is often used in research to assess antimicrobial activity of essential oils (SEMENIUC, 2016; SIENKIEWICZ *et al.*, 2017; MOGHADDAM *et al.*, 2018). Gutiérrez-Pacheco *et al.* (2019) underline that it is one of the main antibiotics used in Latin American countries to control bacterial diseases caused by *Pseudomonas*, *Ralstonia*, and *Xanthomonas* species. Table 2 details the antibacterial activity of gentamicin with their respective inhibition zones for the bacteria evaluated.

Table 2 - Antibacterial activity of the antibiotic gentamicin on phytopathogenic bacteria

Growth inhibition zone* (mm)	
Bacterium	Gentamicin (20µg/mL)
<i>Xanthomonas axonopodis</i> pv. <i>phaseoli</i>	20.67 a
<i>Pectobacterium carotovorum</i> pv. <i>carotovorum</i>	19.4 a
<i>Pseudomonas syringae</i> pv. <i>tomato</i>	20.22 a
<i>Ralstonia solanacearum</i>	11.30 b

Source: Authors' (2021)

Note: * Means followed by the same letter are not significantly different by the Scott-Knott test at 5% probability.

The results of this study demonstrate the low sensitivity of the species to the antibiotic gentamicin, with *R. solanacearum* showing the greatest resistance and differing from the others. Bacteria can express natural resistance of one genus or species to an antibiotic or acquired resistance through mutations. Works of Li and Yu (2015) and

Chahardoli *et al.* (2017) found inhibition zones of 7.66mm for the dose of 10µg and 23.7mm for 20µg of gentamicin, which evidence the resistance of this species to the antibiotic.

As described in the methods section, the evaluation of the antimicrobial activity of essential oils was carried out first by the agar diffusion technique and later by macro and micro dilution tests. Silvestri *et al.* (2010) argued that these tests are not necessarily comparable, since the dilution method best provides quantitative data, while the plate diffusion is a qualitative method. There are several factors that affect the susceptibility of the diffusion and dilution method, thus, information on experimental conditions and strict standardization in carrying out the test are required. The results obtained by each of these methods may differ because of culture conditions, incubation time, temperature, oxygen rate, culture medium, incubation, concentration of tested substances, dispersion and emulsification of agents used in the oil-water emulsion (OSTROSKY *et al.*, 2008; ROMAN *et al.*, 2017).

The evaluations of the control of the microorganisms by the essential oils showed the greatest sensitivity of the bacteria to the different doses of the antibiotic gentamicin as described below:

3.3 Control of *Xanthomonas axonopodis* pv. *phaseoli*

Among the bacterial diseases that affect the bean crop, the common bacterial blight caused by *X. axonopodis* pv. *phaseoli* significantly reduces production. The main control measures have been the use of good quality seeds, resistant cultivars, crop rotation, removal or incorporation of crop residues to the soil, seed and leaf preventive treatment with antibiotics. The use of chemical control for seed surface elimination of the pathogen is effective, however, in cases of internal seed infection this control is difficult (TORRES *et al.*, 2009; BAJPAI *et al.*, 2011; CORRÊA *et al.*, 2017; RDNIÇ *et al.*, 2018).

Because of the problems associated with disease control methods, the use of natural products is a viable alternative to be combined with agronomic practices as a means to minimize the disease. Table 3 presents the antibacterial effect of the essential oils evaluated against the bacteria *Xanthomonas axonopodis* pv. *phaseolis*.

Table 3 - Growth inhibition zone (mm) of the phytopathogenic bacteria *Xanthomonas axonopodis* pv. *phaseoli* by the essential oils of *Elionurus latiflorus* and *Cymbopogon flexuosus*

Essential oil	Essential oil dose (μL)					
	5	10	15	20	30	40
<i>Elionurus latiflorus</i>	51.41 bB	57.31 bB	58.30 bC	82.50 aA	85.16 aA	85.82 aA
<i>Cymbopogon flexuosus</i>	85.81 aA	85.03 aA	85.02 aA	86.83 aA	85.98 aA	90.00 aA
Citral	-	-	-	88.61 a	-	-

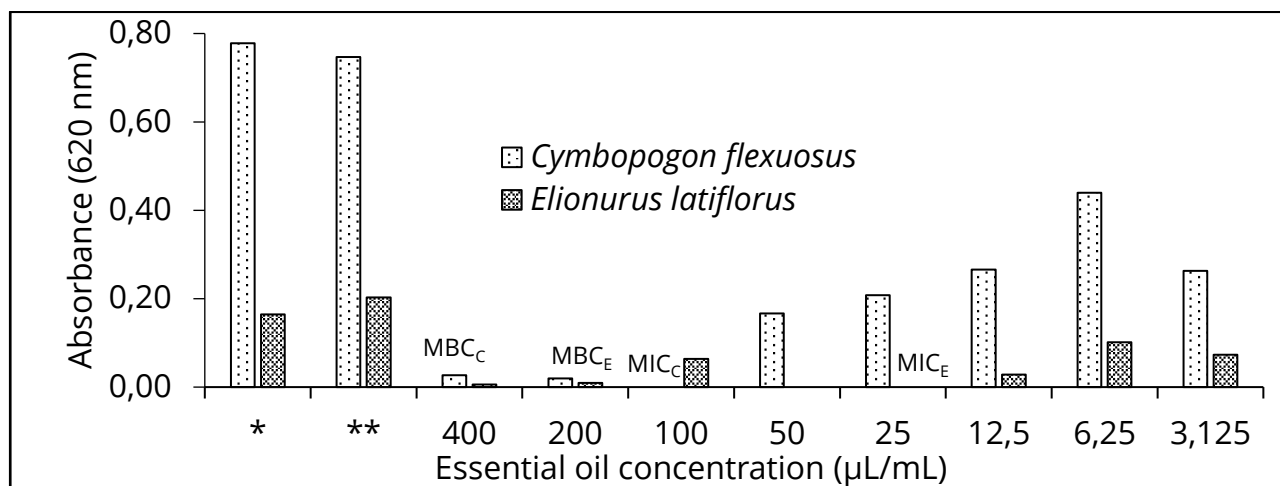
Source: Authors' (2021)

Note: * Means followed by the same letter are not significantly different by the Scott-Knott test at 5% probability.

As can be seen in Table 3, the *C. flexuosus* oil showed a greater control of *X. axonopodis* pv. *phaseolis* at all doses, without significant difference, and being equivalent to the oil of *E. latiflorus* at the highest doses tested (20 μL , 30 μL , 40 μL) that showed no difference. Both oils more effectively inhibited the pathogen compared to gentamicin and had a similar control to pure citral at the dose of 20 μL .

Figure 1 represents MIC and MBC data on the essential oils tested for each bacteria, the data are expressed as the difference between the treatments with the essential oil and the control treatment. It is important to note that the macro and micro dilution tests were initially performed using doses between 0.3125 $\mu\text{L}/\text{mL}$ and 40 $\mu\text{L}/\text{mL}$, but MBC could not be identified for any of the bacteria tested, thus, the dose of the essential oil was increased by 10 times, to between 3.125 $\mu\text{L}/\text{mL}$ and 400 $\mu\text{L}/\text{mL}$.

Figure 1 - Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of *Cymbopogon flexuosus* and *Elionurus latiflorus* essential oils in the control of the phytopathogenic bacteria *Xanthomonas axonopodis* pv. *phaseoli*



Source: Authors' (2021)

Note: * Mueller Hinton (MH). **Mueller Hinton + Dimethylsulfoxide (MH+DMSO).

Figure 1 indicates that for *Cymbopogon flexuosus* essential oil, the MIC_C was 100µL/mL and the MBC_C was 400µL/mL, and for the *Elionurus latiflorus* oil, the MIC_E was 25µL/mL and the MBC_E was 200µL/ml. The control treatments that used Mueller Hinton and Mueller Hinton + DMSO showed no significant difference, demonstrating that DMSO did not interfere with the growth of the bacteria tested throughout the work.

Studies by Gmbh *et al.* (2010), Lucas *et al.* (2012), Todorović *et al.* (2016), Gakuubi (2016), and Popović *et al.* (2018) have proven the effect of different essential oils on the control of species of the genus *Xanthomonas*, but they found no evidence for the bacteria and oils of this study. In the present study, we found that the application of essential oils of *Cymbopogon flexuosus* and *Elionurus latiflorus* provided control of the phytopathogen *Xanthomonas axonopodis* pv. *phaseoli*.

3.4 Control of *Ralstonia solanacearum*

Bacterial wilt caused by *R. solanacearum* is one of the most severe and widespread diseases worldwide since it has a wide host range, geographic reach, and

ability to survive in different environments. Control measures include host resistance, crop rotation, biological soil infestations, and cultural practices. Because of the antibiotic resistance, alternative techniques have been researched, including the use of essential oils such as *Cymbopogon martini*, *Cymbopogon citratus*, *Caryophyllus aromaticus* and *Eucalyptus globulus*, which have shown effectiveness in controlling the pathogen (PARET *et al.*, 2010; CHEN *et al.*, 2014; YULIAR *et al.*, 2015; KARIM *et al.*, 2018).

Table 4 describes the antibacterial effect of the essential oils tested against the bacterium *Ralstonia solanacearum*.

Table 4 - Growth inhibition zone (mm) of the phytopathogenic bacterium *Ralstonia solanacearum* by the essential oils of *Elionurus latiflorus* and *Cymbopogon flexuosus*

Essential oil	Essential oil dose (μL)					
	5	10	15	20	30	40
<i>Elionurus latiflorus</i>	67.12 bC	78.23 aB	85.81 aA	90.00 aA	90.00 aA	90.00 aA
<i>Cymbopogon flexuosus</i>	82.43 aA	82.25 aA	87.87 aA	90.00 aA	86.80 aA	90.00 aA
Citral	-	-	85.72 a	-	-	-

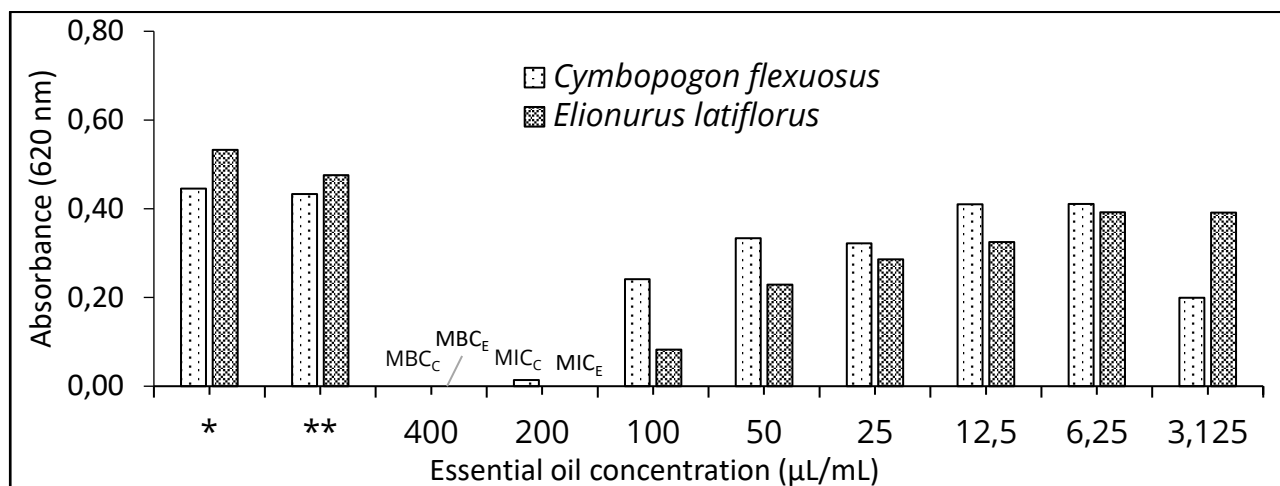
Source: Authors' (2021)

Note: * Means followed by the same letter are not significantly different by the Scott-Knott test at 5% probability.

From Table 4 we can see that the *C. flexuosus* essential oil gave control of *Ralstonia solanacearum* at all doses and showed no significant difference between them. It was equivalent to *E. latiflorus* oil from the dose of 10 μL and showed no significant differences, demonstrating the effectiveness of the oils in the control of the bacterium, with inhibition superior to gentamicin and control similar to pure citral at the dose of 15 μL .

Figure 2 shows the MIC and MBC of the essential oils for *R. solanacearum*. It is observed that for both essential oils the MIC was 200 $\mu\text{L}/\text{mL}$ and the CBM 400 $\mu\text{L}/\text{mL}$.

Figure 2 - Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the essential oils of *Cymbopogon flexuosus* and *Elionurus latiflorus* for the bacterium *Ralstonia solanacearum*



Source: Authors' (2021)

Note: * Mueller Hinton (MH). **Mueller Hinton + Dimethylsulfoxide (MH+DMSO).

Studies carried out on the control of *Ralstonia solanacearum* by essential oils show that the growth inhibiting potential of the oils evaluated in this work is greater than that of the species *Cymbopogon winterianus*, *Rosmarinus officinalis*, *Lippia alba*, *Macleaya cordata* and *Pinus halepensis* (SUENIA *et al.*, 2009; LI and YU, 2014; ASHMAWY *et al.*, 2018).

3.5 Control of *Pectobacterium carotovorum* pv. *carotovorum*

P. carotovorum pv. *carotovorum*, formerly known as *Erwinia carotovora* pv. *carotovora*, affects crops such as potato, carrot, watermelon, cantaloupe, and tomato. Control of this genus depends mainly on bacteriostatic agents, namely hypochlorite, formaldehyde solutions, and antibiotics, due to the ability of this pathogen to adapt to a wide temperature range, which keeps it viable for longer, as well as the saprophytic ability to survive in infected tissues. Research on genetic sequencing, biocontrol, and essential oils has been developed to control this pathogen (GOMES *et al.*, 2005; COSTA *et al.*, 2009; BHAT *et al.*, 2010; LEE *et al.*, 2012; CARVAJAL&VERGARA, 2016; VILLA-RUANO *et al.*, 2017).

Table 5 shows the antibacterial effect of the essential oils tested against the bacterium *Pectobacterium carotovorum* pv. *carotovorum*.

Table 5 - Growth inhibition zone (mm) of the phytopathogenic bacterium *Pectobacterium carotovorum* pv. *carotovorum* by the essential oils of *Elionurus latiflorus* and *Cymbopogon flexuosus*

Essential oil	Essential oil dose (μL)					
	5	10	15	20	30	40
<i>Elionurus latiflorus</i>	69.84 bB	62.53 bB	80.15 aA	81.64 aA	81.75 aA	83.12 aA
<i>Cymbopogon flexuosus</i>	68.98 bB	81.42 aA	81.62 aA	82.45 aA	85.71 aA	90.00 aA
Citral	-	-	84.33 a	-	-	-

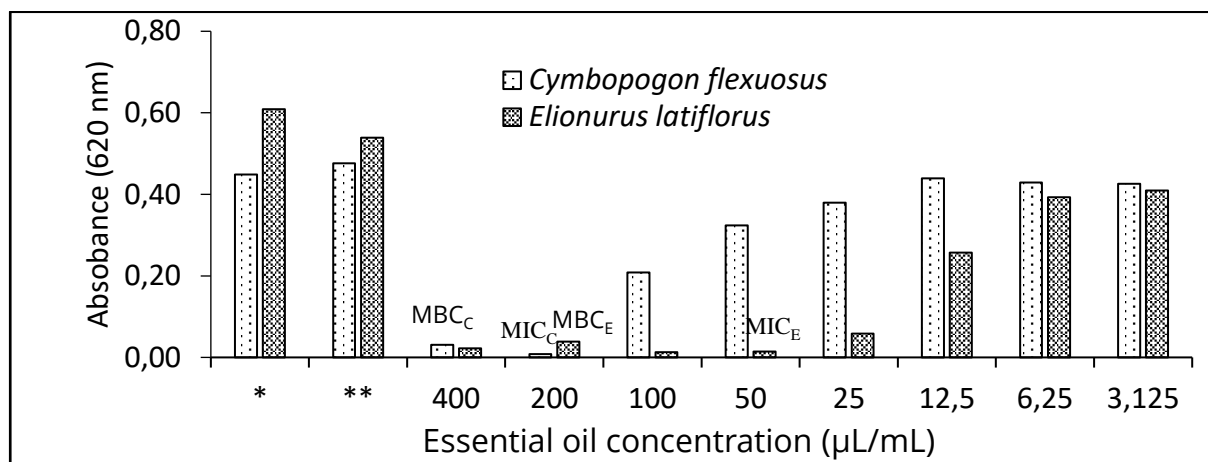
Source: Authors' (2021)

Note: * Means followed by the same letter are not significantly different by the Scott-Knott test at 5% probability.

As detailed in Table 5, the *C. flexuosus* oil provided greater control of *P. carotovorum* pv. *carotovorum* at doses from 10 μL , with no significant differences, and was equivalent to *E. latiflorus* oil from the dose of 15 μL , which were also not significantly different. We found that the essential oils were more efficient in comparison with the control with the antibiotic gentamicin, with bacterium inhibition zone of 19.04mm and control similar to pure citral at the dose of 15 μL .

Figure 3 shows the MIC and MBC of *Cymbopogon flexuosus* and *Elionurus latiflorus* essential oils for *Pectobacterium carotovorum* pv. *carotovorum*, with data representing the difference between the essential oil treatments and the control treatment.

Figure 3 - Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of *Cymbopogon flexuosus* and *Elionurus latiflorus* essential oils for *Pectobacterium carotovorum* pv. *carotovorum*



Source: Authors' (2021)

Note: * Mueller Hinton (MH). **Mueller Hinton + Dimethylsulfoxide (MH+DMSO).

We observe from Figure 3, which represents the difference between treatments with essential oil and control, that for *Cymbopogon flexuosus* essential oil, MIC_C was 200µL/mL and MBC_C was 400µL/mL, and for *Elionurus latiflorus* oil, MIC_E was 50µL/mL and MBC_E was 200µL/mL.

The control of bacteria of the *Pectobacterium* genus showed favorable results in research carried out with the essential oils of *Teucrium montanum*, *Ocimum basilicum*, *Cymbopogon winterianus* and *Curcuma longa* (VUKOVIC, 2007; COSTA *et al*, 2009; HASSAN *et al.*, 2016). Specifically for the variant *carotovorum*, control was achieved with the plant species *Ziziphora persica*, *Cinnamomum zeylanicum* and *Brassica nigra* (OZTURK&ERCISLI, 2006; BRAVO CADENA *et al.*, 2018).

3.6 Control of *Pseudomonas syringae* pv. *tomato*

P. syringae pv. *tomato* causes the important leaf spot disease in tomato, which can bring losses of up to 30% in production, in addition to compromising the quality and commercial value of the fruit. Currently, chemical control is carried out with copper-based bactericides, however, in the last 25 years, a growing number of

publications have reported copper tolerance in *P. syringae* pv. *tomato* and reduced efficacy of substance-based products leading to interest in alternative control strategies (SILVA *et al.*, 2008; GRIF *et al.*, 2017).

Table 6 shows the antibacterial effect of the essential oils tested against the bacteria *Pseudomonas syringae* pv. *tomato*.

Table 6 - Growth inhibition zone (mm) of the phytopathogenic bacterium *Pseudomonas syringae* pv. *tomato* by the essential oils of *Elionurus latiflorus* and *Cymbopogon flexuosus*

Essential oil	Essential oil dose (μL)					
	5	10	15	20	30	40
<i>Elionurus latiflorus</i>	51.48 bB	58.11 bB	63.72 bB	63.88 bB	64.55 bB	87.30 aA
<i>Cymbopogon flexuosus</i>	51.22 bB	58.89 Bb	66.01 bB	67.73 bB	81.63 aA	84.63 aA
Citral	-	-	-	-	-	85.32 a

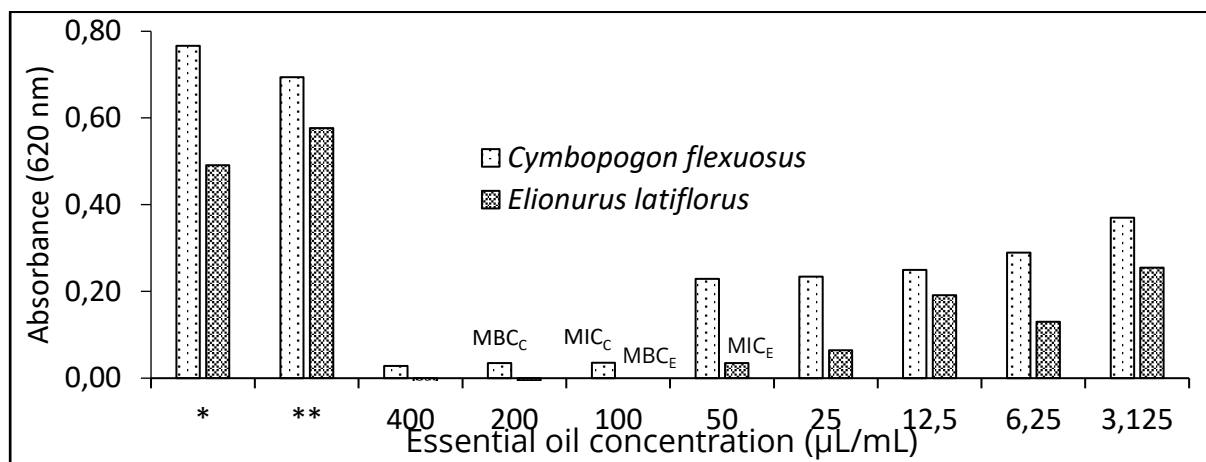
Source: Authors' (2021)

Note: * Means followed by the same letter are not significantly different by the Scott-Knott test at 5% probability.

Among the studied species, *P. syringae* pv. *tomato* showed the greatest resistance to essential oils in the in vitro test. Greater control only occurred at doses 30 μL and 40 μL for *C. flexuosus* and 40 μL for *E. latiflorus*, with inhibition superior to gentamicin at all doses, but with similar control to the pure citral at the dose of 40 μL .

Figure 4 shows the MIC and MBC of *Cymbopogon flexuosus* and *Elionurus latiflorus* essential oils for *Pseudomonas syringae* pv. *tomato*, with data representing the difference between the treatments with the essential oil and the control treatment.

Figure 4 - Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of *Cymbopogon flexuosus* and *Elionurus latiflorus* essential oils for *Pseudomonas syringae* pv. *tomato*



Source: Authors' (2021)

Note: * Mueller Hinton (MH). **Mueller Hinton + Dimethylsulfoxide (MH+DMSO).

As shown in Figure 04, which represents the difference between the treatments with the essential oil and the control treatment, that for the essential oil of *Cymbopogon flexuosus*, the MIC_C was 100µL/mL and the MBC_C was 200µL/mL, and for *Elionurus latiflorus* oil, MIC_E was 50µL/mL and MBC_E was 100µL/mL.

Studies by Lima Guimarães *et al.* (2014) and Villa-Ruano *et al.* (2017) proved the inhibition of the *P. syringae* pv. *tomato* growth with essential oils of *Lippia sidoides* and *Cuscuta mitraeformis*, but with doses higher than those tested in this work.

The results of this study for all bacteria tested corroborate the effectiveness of plants of the *Poaceae* family against microorganisms. These species present several metabolites that exhibit important biological action, showing a promising strategy to reduce the indiscriminate use of synthetic insecticides that have favored the emergence of resistant populations.

These findings revealed that the essential oils have antimicrobial activity in different magnitudes, and the bacteria tested, all Gram-negative, were sensitive to *Cymbopogon flexuosus* and *Elionurus latiflorus* oils.

Studies attribute less effectiveness of essential oils against Gram-negative bacteria due to the greater complexity of its polysaccharide-rich double cell wall, with an outer membrane that prevents the penetration of macromolecules and hydrophobic compounds, in comparison with the simpler structure of the Gram-positive bacterial cell wall (PIERI *et al.*, 2011; MIRANDA *et al.*, 2016; CHOUHAN *et al.*, 2017).

Essential oils and their terpenoid compounds damage biological membranes due to their lipophilic properties, however, the antimicrobial action mechanism cannot be attributed to something specific, considering the variety and amount of chemical compounds present in essential oils and their antibacterial activity. In a broad sense, it is known that the main site of the toxic action of terpenes is the plasma membrane, by its disruption that causes loss of several enzymes and nutrients, however, the final mechanisms of growth inhibition, cell damage, and inactivation are not completely defined. Due to the multicomponent nature of essential oils, bacterial resistance is less likely to develop, as these oils affect numerous targets in pathogens (NAKAMURA *et al.*, 2003; LUO *et al.*, 2004; PARK *et al.*, 2009; HORVÁTH & ÁCS, 2015; SOLIMAN *et al.*, 2017).

Monoterpenes such as the citral are highly hydrophobic and their bactericidal effects are associated with interactions with the cell membrane and, thus, the increase in their absorption by the microorganism. Monoterpenes preferentially tend to move from the aqueous phase towards the membrane structures, which results in their expansion, increased fluidity, and permeability; and then disrupting the embedded membrane proteins, altering the ion transport process, and inhibiting cell respiration. Therefore, the damage caused to the structure of the cytoplasmic membrane lead to impairment of functions such as site of enzymatic action, selective barrier, and energy generation (ONAWUNMI, 1989; TURINA *et al.* 2006; VALERIANO *et al.*, 2012; ROCHA *et al.*, 2014).

The difficulty in comparing the results in the literature is a common problem faced in studying the antimicrobial activity of essential oils and plant-derived products, since there are numerous variations in the methodologies used by different

researchers. This indicates the need for standardized methodologies to assess the antimicrobial activity of essential oils and allow the comparison of results obtained by different authors (SILVEIRA *et al.*, 2012).

The results of this study are encouraging as they show that natural products such as essential oils represent potential sources of bioactive compounds and alternatives for the control of phytopathogenic bacteria. Future studies on these substances are therefore required in order to verify their toxicity, aiming at a possible use of these oils as antimicrobial herbal medicines.

From the foregoing, therefore, essential oils can be an alternative for the control of phytopathogenic bacteria, as they can influence the reduction of pesticide use and are an abundant natural source of molecules, many unknown, which can serve as a model for chemical synthesis and generate low-cost, effective, environmentally safe, standardized, registered, and quality control products aimed at the reproducibility and constancy of chemical components, and, above all, that meet the needs of farmers (CANSIAN *et al.*, 2010; SANTOS *et al.*, 2014; SOUZA *et al.*, 2017).

4 CONCLUSIONS

The major component of the essential oils of *Cymbopogon flexuosus* and *Eliononurus latiflorus* identified was citral, corresponding to 71.6% and 65.38% of the essential oil composition of each species, respectively.

The analysis of the essential oils showed *in vitro* antimicrobial activity against all tested bacteria. The bactericidal effect of both essential oils was superior to that of the antibiotic gentamicin, with inhibition zones between 11.30mm and 20.67mm, while for the tested oils the inhibition zones ranged from 51.22mm to 90mm.

The analyses carried out with pure citral showed inhibition zones similar to those of the essential oils and greater than gentamicin in the *in vitro* tests, with average inhibition zones of 86mm.

Minimal inhibitory concentration (MIC) between 25µL/mL and 200µL/mL and minimal bactericidal concentration (MBC) from 100µL/mL to 400µL/mL were found for the essential oils tested, varying according to the bacterium species.

These results show that the use of natural products, such as essential oils from *Cymbopogon flexuosus* and *Elionorus latiflorus* are an effective alternative for the control of phytopathogenic bacteria *Xanthomonas axonopodis* pv. *phaseoli*, *Ralstonia solanacearum*, *Pectobacterium carotovorum* pv. *carotovorum*, and *Pseudomonas syringae* pv. *tomato* in order to reduce the use of products that may impact health and the environment and combined with other practices in the integrated management of pests and diseases.

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