

## Articles

# Essential oils in the “*in vitro*” control of fungi causing root rot and trunk canker

Óleos essenciais no controle “*in vitro*” de fungos causadores de podridão de raízes e cancro do tronco

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## ABSTRACT

The disease control caused by fungal pathogens is often achieved by using chemicals. However, alternatives that cause less environmental impact should be studied. Thus, essential oils (EOs) are a possibility which have potential for controlling plant diseases. This study aimed to evaluate, *in vitro*, the effects of different concentrations of the essential oils of *Hesperozygis ringens* (Benth.) Epling and *Ocimum gratissimum* L. on the development of *Fusarium oxysporum* and *Fusarium solani* (which cause root rot) and *Neofusicoccum parvum* and *Pseudofusicoccum kimberleyense* (which cause stem canker). For this purpose, the fungi were transplanted onto Potato Dextrose Agar (PDA) culture medium, enriched with three concentrations of each of the EOs previously diluted in ethanol. The plates were incubated in a B.O.D. chamber. (25 °C, 12h photoperiod) for seven days. At the end of the incubation period, mycelial growth, sporulation, size and number of spores were evaluated. Based on the diameters of the colonies on the seventh day, the percentage of inhibition of mycelial growth was calculated. At a concentration of 1.00 µL. mL<sup>-1</sup>, *Ocimum gratissimum* essential oil significantly reduced the final diameter, together with sporulation of the fungal colonies of the isolates. *Hesperozygis ringens* essential oil also effectively controlled the pathogens. mL<sup>-1</sup>. Inhibition of mycelial growth was observed at all concentrations evaluated, proving the efficiency of the use of EOs in reducing the growth of fungal colonies. It is concluded that the essential oils of *Ocimum gratissimum* and *Hesperozygis ringens* present antifungal activity against the fungi *Fusarium oxysporum*, *Fusarium solani*, *Neofusicoccum parvum* and *Pseudofusicoccum kimberleyense*.

**Keywords:** Disease control; *Fusarium oxysporum*; *Fusarium solani*; *Neofusicoccum parvum*; *Pseudofusicoccum kimberleyense*

## RESUMO

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O controle de doenças ocasionadas por patógenos fúngicos é frequentemente realizado com o uso de produtos químicos. No entanto, alternativas que causem menor impacto ambiental devem ser estudadas. Desta forma, têm-se como possibilidade a utilização dos óleos essenciais (OE's), que apresentam potencial para controle de doenças em plantas. Este trabalho teve como objetivo avaliar, *in vitro*, os efeitos de diferentes concentrações dos óleos essenciais de *Hesperozygis ringens* (Benth.) Epling e *Ocimum gratissimum* L. no desenvolvimento dos fungos *Fusarium oxysporum* e *Fusarium solani* (causadores de podridão-de-raízes) e *Neofusicoccum parvum* e *Pseudofusicoccum kimberleyense* (causadores do cancro do tronco). Para tanto, os fungos foram repicados para meio de cultura Batata-Dextrose-Ágar (BDA), enriquecido com três concentrações de cada um dos OE's previamente diluídos em etanol. As placas foram incubadas em câmara do tipo B.O.D. (25 °C, 12h fotoperíodo), por sete dias. Ao final do período de incubação, foram avaliados o crescimento micelial, esporulação, dimensão e número de conídios. Com base nos diâmetros das colônias ao sétimo dia, foi calculado o percentual de inibição do crescimento micelial. Na concentração de 1,00 µL. mL<sup>-1</sup> o óleo essencial de *Ocimum gratissimum* reduziu significativamente o diâmetro final, juntamente com esporulação das colônias fúngicas dos isolados. O óleo essencial de *Hesperozygis ringens* também controlou de forma efetiva os patógenos. Foi observada inibição do crescimento micelial em todas as concentrações avaliadas, comprovando eficiência da utilização dos OE's na redução do crescimento das colônias fúngicas. Conclui-se que os óleos essenciais de *Ocimum gratissimum* e *Hesperozygis ringens* apresentam atividade antifúngica frente aos fungos *Fusarium oxysporum*, *Fusarium solani*, *Neofusicoccum parvum* e *Pseudofusicoccum kimberleyense*.

**Palavras-chave:** Controle de doenças; *Fusarium oxysporum*; *Fusarium solani*; *Neofusicoccum parvum*; *Pseudofusicoccum kimberleyense*

## 1 INTRODUCTION

Currently, nearly 14% of annual crop yield losses are associated with fungal genera, which constitute the largest group of plant pathogens. Studying their control is, therefore, one of the main challenges for achieving global food security and sustainability (Anand; Rajeshkumar, 2022).

In Rio Grande do Sul state, species of the genus *Fusarium* are widely distributed. The isolates found are classified into several species, among which *Fusarium oxysporum* and *Fusarium solani* stand out (Mezzomo, et al., 2021). Some species of this genus are highlighted on the list of pathogens of concern, such as *F. oxysporum*, which spread rapidly throughout Central America due to the replacement of tropical forests by banana (*Musa* sp.) monocultures (Silva, 2022). For forest species, species of the genus

*Fusarium* are reported as one of the main seed-associated pathogens, reducing the germination, causing root rot, and seedling damping-off (Rosário, et al., 2022). In the state of Rio Grande do Sul, a major producer of yerba mate (*Ilex Paraguariensis*), *F. oxysporum* compromises the seedling development, causing root rot, especially in young seedlings grown in nurseries (Quevedo, et al., 2022).

However, *Fusarium solani*, also a cosmopolitan species, attacks the common bean (*Phaseolus vulgaris* L.), cultivated worldwide. The fungus is responsible for hypocotyl rot, seed decomposition, and the seedling damage, both pre- and post-emergence, and can lead to 100% losses in susceptible cultivars (Eke et al., 2020).

Furthermore, from the perspective of economically significant diseases, *Neofusicoccum parvum* is an important pathogen of several plants, causing problems such as brown spot of Chinese walnut (*Myristica* sp.), canker and dieback of redwood (*Sequoiadendron giganteum*), and canker of avocado (*Avocado* sp.), among others (Gou et al., 2023). Pruned or injured trees are vulnerable to infection by *N. parvum*, which can often result in death (Gou et al., 2023). *Pseudofusicoccum kimberleyense* is also a canker-causing pathogen that has been described in eucalyptus (*Eucalyptus* spp.), acacia (*Acacia mangium*), and pine (*Pinus massoniana* Lamb.) (LI et al., 2023) and, more recently, in pecan (*Carya illinoensis*) (Rolim et al., 2020; Rabuske et al., 2023).

The use of chemicals and their adverse environmental consequences has sparked the search for control methods that cause less environmental impact. In this context, pathogen control through secondary compounds present in essential oils (EOs) constitutes a potential means of controlling plant diseases.

Those diseases caused by fungi pose a significant risk to cultivated areas due to the potential for spores to spread from one plant to another. Currently, the control of fungal pathogens is carried out mainly through synthetic fungicides, which, in addition to high costs, offer environmental and toxicological risks, which degrade the environment and put the health of farmers at risk (Sil, et al., 2020).

In this context, the biological properties of EOs have been explored for centuries due to their antiseptic, antioxidant, and anesthetic properties. More recently, EOs have become an important source of biologically active compounds, with antibacterial, fungicidal, herbicidal, and insecticidal properties, among others (Pandey et al., 2017; Madjouko et al., 2019). Integrated agricultural disease management is accelerating the search for methodologies that include biological control, and it is in this context that research on the use of EOs in the control of phytopathogenic fungi is included (Raveau et al., 2020).

*Hesperozygis ringens*, a plant of the Lamiaceae family native to southern Brazil and threatened with extinction, is popularly known as flea repellent (Dolwitsch et al., 2021). Its strong, characteristic odor is easily recognized in the environment, and it is found only in some regions of Rio Grande do Sul state (Leite et al., 2023). In this research, the antifungal activity of *H. ringens* was tested against wood-decaying fungi, such as those that cause white and brown rot. The pulegone component was identified as an inhibitor of the mycelial growth of these fungi (Pinheiro et al., 2021). Therefore, the species has potential for testing to control fungi that cause root rot and canker.

Also from the Lamiaceae family, the genus *Ocimum* comprises over 150 species distributed throughout the tropical region of the world. In certain regions, farmers use plants of this genus to protect tropical fruits against postharvest fungal diseases (Madjouko et al., 2019). The main compounds in *O. gratissimum* EO are thymol, trans-sabinene hydrate, and limonene, which are associated with the fungal cell wall destruction, demonstrating their ability to inhibit the fungal growth (Pedroso et al. 2024). Furthermore, Scariot et al. (2020) used treatments with monoterpenes such as citral, citronellol, and thymol to result in intracellular accumulation of reactive oxygen species (ROS) and in inhibition of the key enzymes.

The evidences presented justify the need to explore EO for the control of these phytopathogenic fungi, which have a wide global distribution and affect a wide range of susceptible commercial cultivars. Thus, the objective of this work was to evaluate the *in*

*in vitro* antifungal activity of essential oils extracted from the species *Hesperozygis ringens* and *Ocimum gratissimum* against phytopathogens that cause root rot in yerba mate (*Fusarium oxysporum* and *Fusarium solani*) and trunk canker in pecan (*Neufusicoccum parvum* and *Pseudofusicoccum kimberleyense*).

## 2 MATERIALS AND METHODS

The EOs of *Ocimum gratissimum* and *Hesperozygis ringens* were provided by the Laboratory of Plant Extracts (Department of Forest Sciences of the Federal University of Santa Maria), where they were stored in amber glass vials and kept in a freezer at -4 °C. Both EOs come from leaves, with the collection site for the plant material of *O. gratissimum* in the municipality of Santa Maria, Rs state (S 29° 41' 3.12"; W 53° 48' 24.84") (Bandeira Jr, et al. 2017), and for *H. ringens*, São Francisco de Assis, Rs state (S 29° 35' 43.1"; W 055° 07' 33.4") (Pinheiro et al., 2016).

The fungi were transferred to Potato Dextrose Agar (PDA) medium, supplemented with three concentrations (1.0, 2.0, and 3.0 µL. mL<sup>-1</sup>) of each of the EOs, previously diluted in ethanol and incorporated into the still-melting culture medium, which was poured into 70-mm Petri dishes. Plates containing only PDA medium were used as a control treatment.

After the culture medium solidified, 7-mm-diameter discs obtained from pure colonies were deposited in the center of the plates. For each treatment, six replicates were performed. The plates were then incubated in a B.O.D. chamber (25 ± 2 °C, 12-h photoperiod) for seven days.

The effect of essential oils on the mycelial growth of fungal isolates was assessed by daily measurements of colony diameter in two opposite directions using a digital caliper, obtaining the average for each replicate. After the incubation period, the percentage of mycelial growth inhibition was calculated according to Quevedo et al. (2024). Furthermore, sporulation, conidial size, number of spores, and mode of action of the oils were characterized.

The experiment was conducted in a completely randomized design, with five treatments and six replicates. The Scott-Knott test was used as a statistical procedure with a 5% probability of error, using the SISVAR statistical analysis program (Ferreira, 2014).

### 3 RESULTS

Analysis of mycelial growth of fungal isolates in PDA medium demonstrated that all presented reduced growth due to *O. gratissimum* essential oil, differing statistically from the control (Table 1).

Table 1 – Mycelial growth and percentage inhibition of pathogenic isolates in Potato-Dextrose-Agar culture medium added with different concentrations of *Ocimum gratissimum* essential oil (EO)

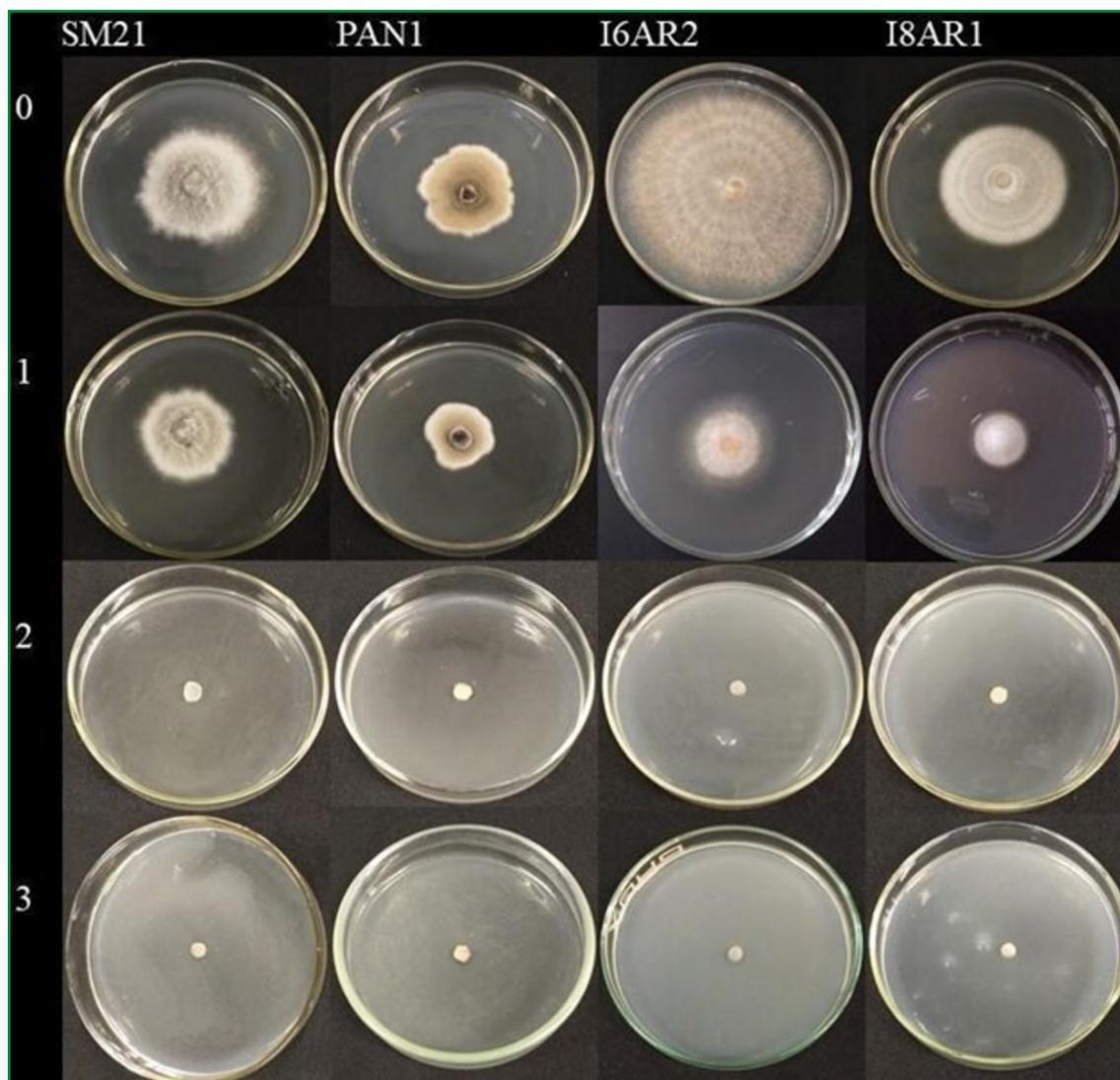
Concentration OE ( $\mu\text{L mL}^{-1}$ )	Mycelial growth (mm)				Inhibition (%)			
	SM21	PAN1	I6AR2	I8AR1	SM21	PAN1	I6AR2	I8AR1
0	46,07 aB*	35,19 aA	79,20 aA	46,98 aB	-	-	-	-
1	6,24 bB	3,97 bB	11,80 bB	22,57 bA	22,76 bB*	37,78 bA	12,22 bC	6,75 bD
2	0,00 bA	0,00 bA	0,00 bA	0,00 cA	100,00 aA	100,00 aA	100,00 aA	100,00 aA
3	0,00 bA	0,00 bA	0,00 bA	0,00 cA	100,00 aA	100,00 aA	100,00 aA	100,00 aA
CV (%)		69,84			2,55			
Mean		15,75			73,46			

Source: Authors (2023)

In where: \* Means followed by the same lowercase letter in the column and uppercase letter in the row do not differ statistically according to the Scott-Knott test at a 5% probability of error; SM21: *Pseudofusicoccum kimberleyense*; PAN1: *Neofusicoccum parvum*; I6AR2: *Fusarium oxysporum*; I8AR1: *Fusarium solani*; CV: Coefficient of Variation.

At concentrations of 2.00 and 3.00  $\mu\text{L mL}^{-1}$ , *O. gratissimum* EO inhibited the mycelial growth of the pathogens by 100%, while the concentration of 1.00  $\mu\text{L mL}^{-1}$  varied its inhibition percentage between 6.75, 12.22, 22.76 and 37.78% for I8AR1, I6AR2, SM21 and PAN1, respectively (Figure 1).

Figure 1 – Colonies of pathogenic fungal isolates after seven days of incubation in Potato-Dextrose-Agar culture medium added with different concentrations of *Ocimum gratissimum* essential oil (EO)



Source: Authors (2023)

In where: SM21: *Pseudofusicoccum kimberleyense*; PAN1: *Neofusicoccum parvum*; I6AR2: *Fusarium oxysporum*; I8AR1: *Fusarium solani*.

Concentrations of 2.00 and 3.00  $\mu\text{L mL}^{-1}$  of *O. gratissimum* EO completely inhibited the mycelial growth of all pathogens, limiting the analysis of morphological characteristics to the control and 1.00  $\mu\text{L mL}^{-1}$  concentrations. Furthermore, despite observing mycelial growth, the isolate SM21 did not exhibit sporulation in any of the treatments evaluated.

For the sporulation variable (Table 2), the results showed statistical differences between the isolates at both concentrations. For isolates PAN1 and I8AR1 at the 1.00  $\mu\text{L mL}^{-1}$  concentration, there was no significant difference compared to the control, which was not the case for the isolate I6AR2, which exhibited lower sporulation at the 1.00  $\mu\text{L mL}^{-1}$  concentration compared to the control.

Table 2 – Sporulation of pathogenic isolates in Potato-Dextrose-Agar culture medium supplemented with 1.00  $\mu\text{L mL}^{-1}$  of *Ocimum gratissimum* essential oil (EO)

Concentration OE ( $\mu\text{L}\cdot\text{mL}^{-1}$ )	Sporulation (conidia. $\text{mL}^{-1}$ )		
	PAN1	I6AR2	I8AR1
0	4,39 $\times 10^5$ aC*	4,01 $\times 10^6$ aA	2,12 $\times 10^6$ aB
1	1,55 $\times 10^5$ aC	2,47 $\times 10^6$ bA	1,04 $\times 10^6$ bB
CV (%)	15,77		
Mean	1,71 $\times 10^6$		

Source: Authors (2023)

In where: \* Means followed by the same lowercase letter in the column and uppercase letter in the row do not differ statistically according to the Scott-Knott test at a 5% probability of error; PAN1: *Neofusicoccum parvum*; I6AR2: *Fusarium oxysporum*; I8AR1: *Fusarium solani*; CV: Coefficient of Variation.

Regarding the size of the conidia (Table 3), a reduction in both length and width was observed. The three pathogens that sporulated at a concentration of 1.00  $\mu\text{L mL}^{-1}$  presented smaller conidia compared to the control treatment.

Table 3 – Length and width of conidia of pathogenic isolates in Potato-Dextrose-Agar culture medium added with 1.00  $\mu\text{L mL}^{-1}$  of *Ocimum gratissimum* essential oil (EO)

Concentration OE ( $\mu\text{L}\cdot\text{mL}^{-1}$ )	Length ( $\mu\text{m}$ )			Width ( $\mu\text{m}$ )		
	PAN1	I6AR2	I8AR1	PAN1	I6AR2	I8AR1
0	7,65 aC*	8,64 aB	10,31 aA	4,22 aB*	3,60 aB	5,66 aA
1	6,50 bB	6,19 bB	9,02 bA	2,86 bB	2,87 bB	3,58 bA
CV (%)	6,59			11,21		
Mean	8,05			3,8		

Source: Authors (2023)

In where: \* Means followed by the same lowercase letter in the column and uppercase letter in the row do not differ statistically according to the Scott-Knott test at a 5% probability of error; PAN1: *Neofusicoccum parvum*; I6AR2: *Fusarium oxysporum*; I8AR1: *Fusarium solani*; CV: Coefficient of Variation.

When observing the mycelial growth of the isolates cultivated in PDA culture medium (Table 4), it was found that all presented reduced growth due to the action of *H. ringens* EO, differing statistically from the control. The concentrations of 2.00 and 3.00  $\mu\text{L} \cdot \text{mL}^{-1}$  resulted in a higher percentage of inhibition when compared to the concentration of 1.00  $\mu\text{L} \cdot \text{mL}^{-1}$  for the three isolates.

Table 4 – Mycelial growth and percentage inhibition of pathogenic isolates in Potato-Dextrose-Agar culture medium added with different concentrations of essential oil (EO) of *Hesperozygis ringens*

Concentration OE ( $\mu\text{L} \cdot \text{mL}^{-1}$ )	Mycelial growth (mm)				Inhibition (%)			
	SM21	PAN1	I6AR2	I8AR1	SM21	PAN1	I6AR2	I8AR1
0	65,48 aA*	34,66 aC	62,74 aA	52,73 aB				
1	27,13 bB	22,30 bB	15,81 bC	36,98 bA	58,57 cB*	35,68 bC	74,81 bA	29,87 cC
2	18,66 cA	4,31 cB	0,00 cB	19,74 cA	71,51 bB	87,59 aA	100,00 aA	62,58 bB
3	0,00 dA	1,61 cA	0,00 cA	4,11 dA	100,00 aA	95,36 aA	100,00 aA	92,21 aA
CV (%)		27,9				14,05		
Média		22,89				75,68		

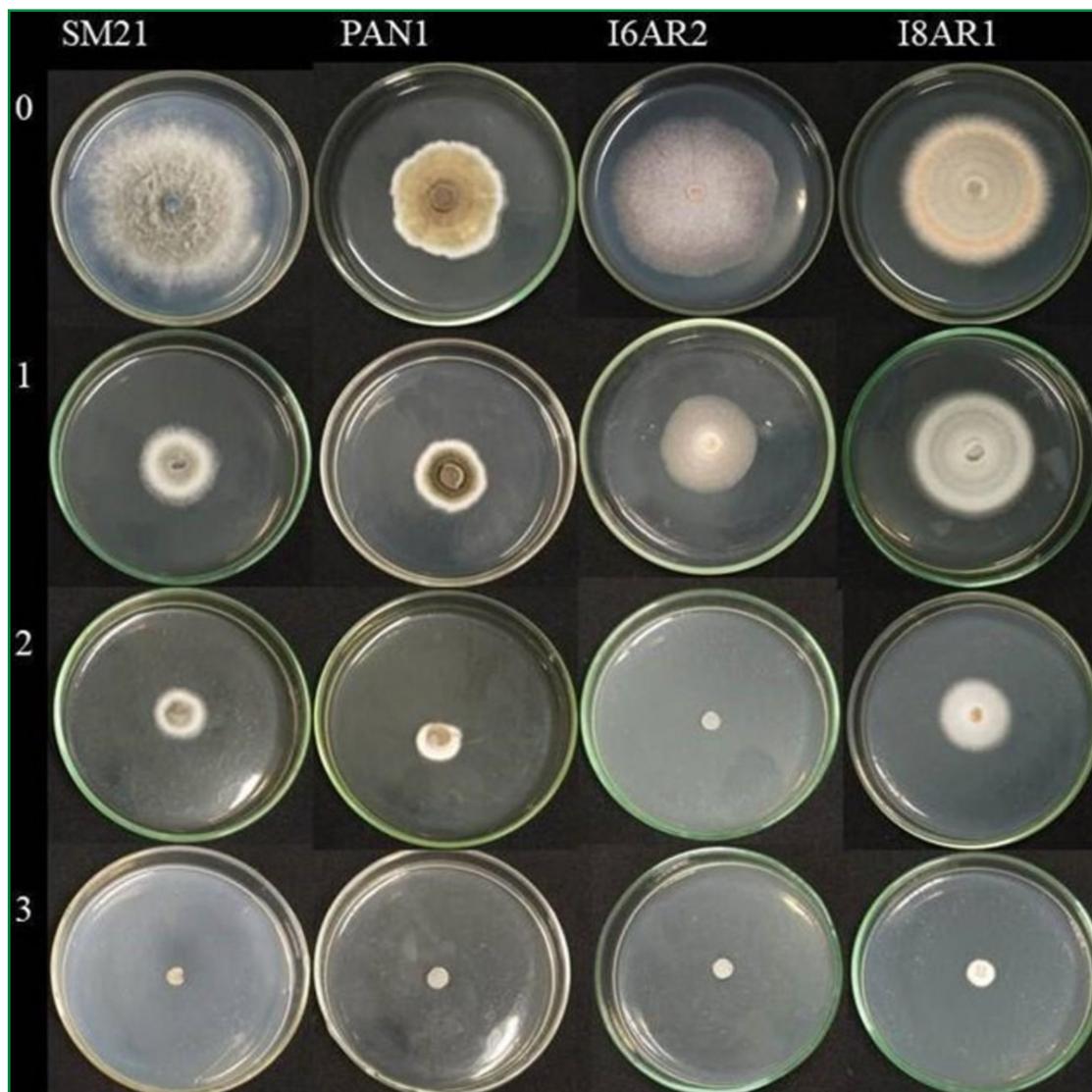
Source: Authors (2023)

In where: \* Means followed by the same lowercase letter in the column and uppercase letter in the row do not differ statistically according to the Scott-Knott test at a 5% probability of error; SM21: *Pseudofusicoccum kimberleyense*; PAN1: *Neofusicoccum parvum*; I6AR2: *Fusarium oxysporum*; I8AR1: *Fusarium solani*; CV: Coefficient of Variation.

At concentrations of 2.00 and 3.00  $\mu\text{L} \cdot \text{mL}^{-1}$  there was a higher inhibition of *H. ringens* essential oil for all isolates, differing statistically from the concentration of 1.00  $\mu\text{L} \cdot \text{mL}^{-1}$ , while it varied in the percentage of inhibition between the isolates (Figure 2).

The 3.00  $\mu\text{L} \cdot \text{mL}^{-1}$  concentration of *H. ringens* EO was most effective in reducing the growth of all isolates. Since fungal colonies grew at different concentrations (0, 1, 2, and 3  $\mu\text{L} \cdot \text{mL}^{-1}$ ), it was necessary to analyze the morphological characteristics of the colonies to which the different EO concentrations were applied. Furthermore, the isolate SM21 did not exhibit the sporulation.

Figure 2 – Colonies of pathogenic fungal isolates after seven days of incubation in Potato-Dextrose-Agar culture medium added with different concentrations of essential oil (EO) of *Hesperozygis ringens*



Source: Authors (2023)

In where: SM21: *Pseudofusicoccum kimberleyense*; PAN1: *Neofusicoccum parvum*; I6AR2: *Fusarium oxysporum*; I8AR1: *Fusarium solani*.

Observing the results in Table 5, with the sporulation variable, the results showed statistical differences between the isolates at these concentrations. For the isolate PAN1 at concentrations of 1.00, 2.00, and 3.00  $\mu\text{L mL}^{-1}$ , there was no significant difference compared to the control, which was not the case for the isolate I6AR2, which had lower sporulation at these concentrations. For the isolate I8AR1, the largest number of spores was identified at the 1.00  $\mu\text{L mL}^{-1}$  concentration compared to the control.

Table 5 – Sporulation of pathogenic isolates in Potato-Dextrose-Agar culture medium added with different concentrations of essential oil (EO) of *Hesperozygis ringens*

Concentration OE (µL·mL <sup>-1</sup> )	Sporulation (conidia. mL <sup>-1</sup> )		
	PAN1	I6AR2	I8AR1
0	4,39x10 <sup>5</sup> aC*	4,01x10 <sup>6</sup> aA	2,12x10 <sup>6</sup> cB
1	2,04x10 <sup>5</sup> aB	1,83x10 <sup>5</sup> bB	4,39x10 <sup>6</sup> aA
2	1,62x10 <sup>5</sup> aB	0,00 bB	3,34x10 <sup>6</sup> bA
3	5,30x10 <sup>4</sup> aA	0,00 bB	5,06x10 <sup>5</sup> dA
CV (%)	54,72		
Média	1,28x10 <sup>6</sup>		

Source: Authors (2023)

In where: \* Means followed by the same lowercase letter in the column and uppercase letter in the row do not differ statistically according to the Scott-Knott test at a 5% probability of error; PAN1: *Neofusicoccum parvum*; I6AR2: *Fusarium oxysporum*; I8AR1: *Fusarium solani*; CV: Coefficient of Variation.

Regarding conidial dimensions (Table 6), there was a reduction in width for all isolates. Regarding length, PAN1 and I6AR2 showed the most significant reduction. The three pathogens that sporulated at the different concentrations showed significantly smaller conidia, both in length and width, when compared to the control treatment. The exception was isolate I8AR1, which showed an increase in spore length at concentrations of 1.00 and 2.00 µL mL<sup>-1</sup>.

Table 6 – The length and width of conidia of pathogenic isolates in Potato-Dextrose-Agar culture medium added with different concentrations of essential oil (EO) of *Hesperozygis ringens*

Concentration OE (µL·mL <sup>-1</sup> )	Length (µm)			Width (µm)		
	PAN1	I6AR2	I8AR1	PAN1	I6AR2	I8AR1
0	8,47 aB*	8,64 aB	10,31 bA	4,22 aB*	3,60 aC	5,66 aA
1	7,55 bB	6,33 bC	11,16 aA	3,61 bB	3,39 aB	5,13 bA
2	7,66 bB	0,00 cC	11,79 aA	4,40 aB	0,00 bC	5,53 aA
3	7,94 bB	0,00 cC	10,56 bA	3,53 bB	0,00 bC	4,80 bA
CV (%)	6,13			7,95		
Média	7,54			3,66		

Source: Authors (2023)

In where: \* Means followed by the same lowercase letter in the column and uppercase letter in the row do not differ statistically according to the Scott-Knott test at a 5% probability of error; PAN1: *Neofusicoccum parvum*; I6AR2: *Fusarium oxysporum*; I8AR1: *Fusarium solani*; CV: Coefficient of Variation.

## 4 DISCUSSION

In general, the essential oils of *Ocimum gratissimum* and *Hesperozygis ringens* were highly effective in reducing mycelial growth of the studied pathogens, with a high percentage of inhibition, significantly different from the control. This effect can be attributed to the constituents of the essential oils, as terpenes and terpenoids are primarily responsible for the high antifungal and antibacterial activity (Mendes et al., 2018; Kumar et al., 2019).

*In vitro* studies with *O. gratissimum* compared to species of the genus *Fusarium* showed a high inhibitory effect of the EO on fungal growth, demonstrating that *O. gratissimum* EO strongly influences the development of *Fusarium oxysporum*, with the growth inhibition ranging from 29.9% to 59.7% (Jeewon et al., 2024). EOs from other species of the genus *Ocimum* were used for *in vitro* tests with different *Fusarium* species, where it was verified, as in the present study, that the inhibition of mycelial growth of the isolates only occurs at higher concentrations (Fontana et al., 2020). In this study, the growth of *F. oxysporum* and *F. solani* was observed at a concentration of 1.00  $\mu\text{L mL}^{-1}$ , but not at 2.00  $\mu\text{L mL}^{-1}$ . In the study by Fontana et al. (2020), it was verified that, at concentrations of 1.00 and 2.00  $\mu\text{L mL}^{-1}$ , the EO of *Ocimum americanum* promoted 100% control over different *Fusarium* species. However, even though it is from the same genus, *O. basilicum*, it required 6.0  $\mu\text{L mL}^{-1}$  to inhibit the growth of *F. solani*, while the *O. gratissimum* oil used in this study inhibited 100% of the mycelial growth of the pathogens at a concentration of 2.00  $\mu\text{L mL}^{-1}$ , indicating its high control potential.

Although a statistical difference was observed between the sporulation of the isolates at both *O. gratissimum* concentrations, this may be attributed to variations in the growth of the isolates during the incubation period. Regarding the sporulation with *O. gratissimum* EO, only the isolate I6AR2 showed a reduction, which differs from the results of Irandegani et al. (2023), who observed in *in vitro* tests that *Satureja hortensi* EO produced the maximum inhibitory effect on *Fusarium cugenangense* sporulation.

Regarding the inhibition of mycelial growth, Pinheiro et al. (2021) found that *H. ringens* EO significantly impaired the development of the wood-rotting fungus *Ganoderma applanatum* at a concentration of 1.25  $\mu\text{L mL}^{-1}$ . This result is similar to that found in this study, where high inhibition percentages occurred at a concentration of 2.00  $\mu\text{L mL}^{-1}$ . This occurs due to the interaction of monoterpenes, the main component of many oils, with the fungal cell membrane, which disrupts cellular and energy homeostasis, leading to cell membrane damage and metabolic changes (PEDROSO, et al., 2024).

However, when reinoculating the mycelium discs, fungal growth was observed after seven days, characterizing the fungistatic activity of this EO. The results of *in vitro* tests indicate high antifungal activity for *H. ringens* EO, and the effect was detected primarily at the same concentration as the conventional antifungal agent used as a positive control (Pinheiro, et al., 2021). When in contact with isolates I8AR1 and PAN1, the EO of *H. ringens* did not have a significant influence, unlike previous studies, where some EOs, such as *Satureja hortensis*, demonstrate an inhibitory effect on *F. oxysporum* sporulation, reducing sporulation by 100% at a concentration of 1.0  $\mu\text{L mL}^{-1}$ . (Irandegani, et al., 2023). Kryzsko-Lupicka et al. (2019) reported that thyme (*Thymus vulgaris* L.) monoterpenoids had a maximum inhibitory effect on the growth and sporulation of *Fusarium* sp. However, isolate I8AR1 did not show the same response. Isolate I6AR2 was the only one that showed a reduction in the number of conidia.

The isolates I8AR1, I6AR2, PAN1, and SM21 from this experiment had their development affected by the EOs of *O. gratissimum* and *H. ringens*, as in the study by Pinheiro et al. (2021), where three of the fungal species evaluated were not completely eliminated by the EO at the concentrations tested, but had their development affected.

## 5 CONCLUSIONS

The essential oils of *Ocimum gratissimum* and *Hesperozygis ringens* have *in vitro* antifungal activity against the fungi *Neofusicoccum parvum*, *Fusarium oxysporum*, and *Fusarium solani*, with the concentration used influencing final colony diameter and sporulation.

For *Pseudofusicoccum kimberleyense*, which did not sporulate, a concentration of 1.00  $\mu\text{L mL}^{-1}$  of EOs negatively affects colony diameter, while higher concentrations, such as 2.00 and 3.00  $\mu\text{L mL}^{-1}$ , inhibit the mycelial growth.

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