IN VITRO ANTIFUNGAL ACTIVITY OF *Myrcia ovata* ESSENTIAL OILS AND THEIR MAJOR COMPOUNDS AGAINST PATHOGENS OF CITRUS, SWEET POTATO, AND COCONUT

ATIVIDADE ANTIFUNGICA IN VITRO DE ÓLEOS ESSENCIAIS DE Myrcia ovata *E SEUS COMPOSTOS MAJORITÁRIOS SOBRE PATÓGENOS DE CITROS, BATATA-DOCE E COQUEIRO*

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ABSTRACT: *Myrcia ovata*, an endemic species to the Brazilian Atlantic Forest, presents antifungal properties. The phytopathogens *Colletotrichum acutatum, Plenodomus destruens*, and *Thielaviopsis paradoxa* are responsible for the diseases citrus postbloom fruit drop, sweet potato foot rot, and *coconut* stem bleeding, respectively. The antifungal activity of the essential oils of five *M. ovata* chemotypes (MYRO-159, nerolic acid chemotype; MYRO-180, nerolic acid + linalool chemotype; MYRO-388, geraniol chemotype; MYRO-157, citral + (*E*)-nerolidol chemotype; and MYRO-174, isopulegol + linalool chemotype), four major compounds (nerolic acid, nerolic acid + linalool, geraniol, and citral + (*E*)-nerolidol), and three pure compounds (citral, (*E*)-nerolidol, and linalool) against the fungi *C. acutatum, P. destruens*, and *T. paradoxa* were evaluated. For this, *in vitro* tests were conducted in a completely randomized design with three replications, testing concentrations (*v*/*v*) ranging from 0.01 to 1.0 $\mu L.mL^{-1}$. All treatments presented toxicity at different levels to the three fungi. For *C. acutatum*, the essential oil from the individual MYRO-180 (nerolic acid + linalool chemotype) and its major compound showed the lowest Minimal Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC) of 0.03 and 0.1 $\mu L.mL^{-1}$, respectively. *For P. destruens, the essential oil from the individual MYRO-159 (nerolic acid* chemotype) *presented the lowest MIC of 0.05* $\mu L.mL^{-1}$. *The nerolic acid + linalool chemotype and its major compound presented an MFC of 0.07* $\mu L.mL^{-1}$. *For T. paradoxa, the major compound citral + (E)-nerolidol stood out with the lowest MIC and MFC of* 0.03 and 0.2 $\mu L.mL^{-1}$, respectively. *Linalool presented the lowest toxicity to the three tested fungi.*

KEYWORDS: Myrtaceae. *Colletotrichum acutatum*. *Plenodomus destruens*. *Thielaviopsis paradoxa*.

INTRODUCTION

the phylum Ascomycota, are responsible for economic losses in planted forests and crops. The fungi *Colletotrichum acutatum* J.H. Simmonds, *Plenodomus destruens* Hater, and *Thielaviopsis paradoxa* (De Seynes) Höhn. can attack plants anytime during the production cycle, especially at the pre-harvest stage. They are respectively responsible for the diseases citrus postbloom fruit drop, sweet potato foot rot, and *coconut* stem bleeding.

In periods of mild temperature and high humidity, which favor the permanence of wet leaves, the losses caused by citrus postbloom fruit drop can reach 80% of the production (GOES et s same percentage of loss is detected in crops infected with sweet potato foot rot, especially when using repeated crops in the same area, with two or three harvests per year. For some authors, it is considered as the main fungal disease in sweet potatoes (PEREIRA et al., 2011; FERNANDES, 2013).

Coconut stem bleeding, caused by *T. paradoxa,* is considered as one of the major diseases in coconut palm trees due to its rapid spread and association with the weevil *Rhynchophorus palmarum* L., which is also associated with *Bursaphelenchus cocophilus*.

These three pathogens together can cause the death of the infected plant after three to four months of the initial symptom, leading to the need of eradication, usually performed by the removal and burning of plants (FONTES et al., 2009; WARWICK; PASSOS, 2009; JESUS; NAVICKIENE, 2014).

Orange, coconut, and sweet potato are species of major economic importance for Brazil. The agricultural production of orange and coconut in the year of 2017 was of 17,304,305 and 1,737,236 tons, respectively, occupying the seventh and sixteenth position of the most productive crops in the country (IBGE, 2017). Sweet potato production in the year of 2016 was of 669,454 tons, occupying, together with peanuts, mandarin, and passion fruit, the 31st position of the most productive crops in the country (IBGE, 2016).

In 2017, the state of São Paulo registered the highest orange production, accounting for 75% of the Brazilian harvest, followed by the states of Bahia and Sergipe, in the second and third places, respectively. For coconut, the northeast region of Brazil accounted for 82% of the production, being the state of Sergipe the third major producer, after Bahia and Ceará (IBGE, 2017). For sweet potato, the state of Rio Grande do Sul was the largest national producer, followed by São Paulo, Minas Gerais, Paraná, and Sergipe (IBGE, 2016). This crop production has increased due to its use as biofuel for ethanol production (BLANK et al., 2017).

According to the Brazilian Ministry of Agriculture, Livestock, and Food Supply – MAPA (2018), no synthetic chemical compound to combat sweet potato foot rot and *coconut* stem bleeding diseases has been registered yet. Furthermore, systemic fungicides of the chemical groups benzimidazole, triazole, and carboxamides are registered for citrus postbloom fruit drop disease. However, some studies have reported the unauthorized use of the fungicide thiophanate-methyl in coconut crops to control stem bleeding, despite being authorized to control *T. paradoxa* in sugarcane crops.

Regarding the use of essential oils as an alternative fungi control method, *C. acutatum* is sensitive to the compounds thymol, carvacrol, citral, and linalool (PÉREZ-SÁNCHEZ et al., 2007; ÖZEK et al., 2010; NUMPAQUE et al., 2011); and *T. paradoxa* is sensitive to the compounds thymol, carvacrol, geraniol, and 1,8-cineole (RAO & SINGH, 1994; CARVALHO et al., 2013). No information has been found on the use of essential oils in the control of *P. destruens*.

Myrcia ovata Cambess (Myrtaceae) is an endemic species to the Brazilian Atlantic Forest, commonly known as *Laranjinha-do-mato*. It is used in folk medicine against diarrhea and colic and has shown to have pesticidal and microbiological action. Concerning the antifungal properties, essential oils of the nerolic acid, nerolic acid + linalool, geraniol, citral, and isopulegol and/or nerolic acid and/or linalool chemotypes have demonstrated actions against *Fusarium solani* (SAMPAIO et al., 2016) and *Lasiodiplodia theobromae* (BLANK et al., 2015), which are both destructive phytopathogenic fungi.

The use of natural products, which are less harmful to the environment and humans, instead of synthetic chemical components has been the subject of scientific papers (GARCIA et al., 2018). This research is justified by the economic importance of orange, coconut, and sweet potato crops; the damage caused by the above-mentioned diseases; the lack of studies on the properties of essential oils or major compounds against *P. destruens*; and the sensitivity of *C. acutatum* and *T. paradoxa* to the compounds present in *M. ovata* essential oils.

Therefore, this study aimed to evaluate the antifungal properties of the essential oils of five *M. ovata* chemotypes, four major compounds, and three pure compounds and determine their Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC) in relation to these fungi.

MATERIAL AND METHODS

Plant material

Myrcia ovata Cambess. leaves were collected from individuals found in a natural population in a village called Sapucaia, in the municipally of Japaratuba, state of Sergipe, Brazil (Figure 1 and Table 1).

The individuals are located in an area of the Brazilian Atlantic Forest, with the predominance of sandy coastal dune vegetation (restinga phytophysiognomy). The shrubs were set in lower vegetation (about 3m high) and spaced on sandy soil. The trees were located at not more than 3km away from the shrubs, in higher (above 25m in height) and dense vegetation, just on the edge of a road, in a soil rich in organic matter, with the presence of litter.

The village is located in the Japaratuba basin region, where the average temperature is 25.3°C and the annual average rainfall is 1.400mm. The rain season occurs between March and August, and the climate is dry sub-humid mesothermal type (SERGIPE, 2000). Agricultural

cultivation is predominant in this region (SERGIPE, 2014).

Leaves were collected on November 23, 2013, and April 24, 2015, during the morning shift. Individuals were not blooming or fruiting, nor was any third of the crown preferred during leaf collection. The twigs, containing leaves, were stored in plastic bags at room temperature and taken to the breeding laboratory of the Federal University of Sergipe, campus of Aracaju. Afterward, twigs were defoliated, and leaves were stored in paper bags, each one containing 75g, and maintained at room temperature until dried in a kiln for five days at 40°C. After that, as soon as possible, the material stored in the same bags at room temperature was used for the essential oil extraction.

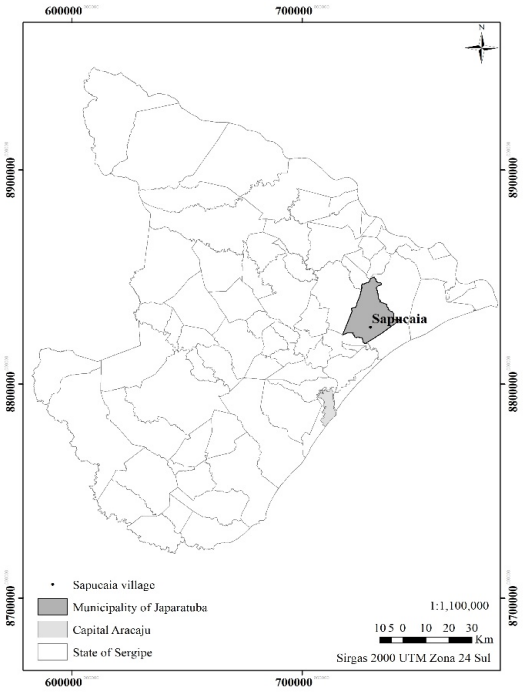

Figure 1. Map of the location of the *Myrcia ovata* individuals in the municipally of Japaratuba, in the state of Sergipe, Brazil.

Table 1. Geographic coordinates of *Myrcia ovata* individuals representative of the five chemotypes, located in the municipally of Japaratuba, in the state of Sergipe, Brazil

Individuals (chemotypes)	Longitude	Latitude	Description	Collection day	Voucher number (Herbarium of the UFS)
MYRO-159 (nerolic acid)	10°37'37.2" S	36°53'17.4" W	Shrub	04/24/2015	ASE 33853
MYRO-180 (nerolic acid + linalool)	10°38'45.3" S	36°52'16.4" W	Tree	11/29/2013	ASE 30879
MYRO-388 (geraniol)	10°38'45.5" S	36°52'16.3" W	Tree	11/29/2013	ASE 33834
MYRO-157 (citral + (*E*)-nerolidol)	10°37'39.0" S	36°53'19.7" W	Shrub	04/24/2015	ASE 33841
MYRO-174 (isopulegol + linalool)	10°38'45.3" S	36°52'17.0" W	Shrub	11/29/2013	ASE 35709

Extraction and characterization of essential oils

The essential oils were extracted by hydrodestillation with a modified Clevenger, kept in 5-ml brown amber jars, closed with lid and bung, and stored in a freezer (-20°C). The essential oils were previously identified in triplicates, and their chemical composition was obtained by GC analyses, using gas chromatography coupled with mass spectrometry and flame ionization detection (GC–MS/FID; QP2010 Ultra, Shimadzu Corporation, Kyoto, Japan), equipped with an autosampler AOC-20i (Shimadzu). The compounds were identified by comparing the observed mass spectra and retention

indices via spectra with the retention indices of Adams (2007), as described by Sampaio et al. (2016).

Antifungal properties

Fungi samples of *C. acutatum*, *P. destruens*, and *T. paradoxa* were obtained from the mycology collection of Embrapa Tabuleiros Costeiros. In this research, the essential oils used were extracted from the plants MYRO-159, MYRO-180, MYRO-388, MYRO-157, and MYRO-174, which had been respectively characterized as nerolic acid, nerolic acid + linalool, geraniol, neral + geranial (which form the citral compound), and isopulegol + linalool chemotypes (see their chemical characterization in Table 2). In addition to the essential oils, the pure compounds citral, (*E*)-nerolidol, and linalool were tested against the mentioned fungi.

Nerolic acid was isolated in the laboratory of organic chemistry of the Federal University of Sergipe (SAMPAIO, 2017). All the other compounds were purchased from the company SIGMA-ALDRICH. To obtain the two major compounds nerolic acid + linalool and citral + (*E*)-nerolidol, the mixture of linalool with nerolic acid and citral with (*E*)-nerolidol were proportionally diluted to the percentage of each compound existing in the respective essential oil chemotypes. The same process was used to obtain the percentage of pure compounds (Table 2).

The essential oils and the major compounds were tested at concentrations ranging from 0.01 to 1.0 $\mu L.mL^{-1}$ to obtain their Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC). Both were initially diluted in 500 µL of DMSO (Dimethyl Sulfoxide PA/ACS, NEON) and then added to the culture medium, which consisted of liquid PDA [1.9 mg of Potato Dextrose Agar (SIGMA-ALDRICH) for each 50 ml of distilled water].

The culture medium containing the chemotypes/compounds was poured into Petri dishes of 9cm diameter. After solidification, a disc of seven millimeters in diameter containing mycelia of the fungus was superimposed in the center of each dish. After inoculation, the Petri dishes were kept in a B.O.D with a 12-hour photoperiod and an average temperature of 25 ± 2°C.

The experimental design was completely randomized. For each chemotype/compound and each concentration, three replications were performed, each one consisting of an individual Petri dish. As for the controls, Petri dishes containing only the BDA culture medium and containing BDA + DMSO were used.

For each fungus, the first incubation period was defined as the time required for the control dishes to present maximum mycelial growth. Therefore, the first incubation period for *C. acutatum*, *P. destruens*, and *T. paradoxa* were nine, seven, and three days, respectively. For the first and second, fungi growth was evaluated every 24 hours, and for the third, every six hours, by measuring the mycelial diameter (mean of two diametrically opposite measures), using a pachymetry.

After the first incubation period, the mycelial discs in which no growth was observed were transferred to new dishes containing only the BDA culture medium. They were incubated for 96 hours (second incubation time) to verify whether such concentration had a fungistatic or fungicidal action. The MIC corresponded to the lowest concentration of chemotype/compound tested in which no mycelial growth was observed only in the first incubation period. The MFC corresponded to the lowest concentration in which no mycelial growth was observed in the two incubation periods.

From the measurements of mycelial growth obtained in the first incubation period, the Percentage of mycelial growth inhibition (PGI) was calculated according to the formula: PGI = [control diameter − treatment diameter)/(control diameter)] × 100 (VINCENT, 1947). PGI data were presented with the mean ± standard error of the mean. From the first incubation period, graphs of the mycelial growth data were generated by the software GraphPad Prism version 6 (GraphPad Software, San Diego, CA - USA), using only the recorded concentrations lower and equal to the MIC.

Table 2. Percentage of chemical compounds content of the essential oils of five *Myrcia ovata* chemotypes from Sergipe, Brazil (SAMPAIO et al., 2016).

Individuals (chemotypes)	C1	C2	C3	C4	C5	C6	C8	C9	C10	C11	C12	C13	C14	C15	C16	C17	C18	C19	C20	C21	C22	C23	C24	C25	C26	C27	C28
MYRO-159 (Nerolic acid)	-	0.26	-	3.98	1.40	-	-	0.72	1.64	-	-	-	-	-	1.28	-	**74.0**	1.63	1.29	-	-	0.50	2.05	-	0.27	5.38	-
MYRO-180 (Nerolic acid + linalool)	0.13	0.24	-	1.14	**25.1**	-	-	0.30	2.05	-	0.32	0.33	1.43	-	1.71	-	**54.0**	0.98	0.39	-	-	5.65	0.30	1.03	-	-	-
MYRO-388 (Geraniol)	0.11	0.10	-	6.67	10.2	-	-	0.63	1.70	-	-	**73.6**	-	1.79	-	-	-	-	0.23	0.52	0.54	-	0.27	-	-	-	-
MYRO-157 (Citral + (*E*)-nerolidol)	0.35	0.50	0.28	4.36	5.77	-	-	1.76	1.38	-	**18.2**	1.87	-	**37.0**	-	-	-	-	1.29	-	-	**20.2**	0.80	-	-	-	-
MYRO-174 (Isopulegol + linalool)	0.86	1.04	0.66	2.88	**19.6**	**27.5**	10.3	2.71	2.33	2.71	0.15	0.45	-	0.10	0.10	0.71	6.33	-	1.19	1.68	1.40	0.29	1.92	-	-	5.69	0.43

Compounds: (C1) α-pinene, (C2) β-pinene, (C3) *p*-cymene, (C4) 1,8-cineole, (C5) linalool, (C6) isopulegol, (C7) citronellal, (C8) *iso*-isopulegol, (C9) terpinen-4-ol, (C10) α-terpineol, (C11) citronellol, (C12) neral, (C13) geraniol, (C14) methyl citronellate, (C15) geranial, (C16) methyl nerolate, (C17) citronellic acid, (C18) nerolic acid, (C19) geranic acid, (C20) (E)-caryophyllene, (C21) β-selinene, (C22) α-selinene, (C23) (*E*)-nerolidol, (C24) caryophyllene oxide, (C25) β-bisabolol, (C26) farnesol (2E, 6Z), (C27) farnesol (2Z, 6E), (C28) farnesol (2E, 6E).

RESULTS

In vitro antifungal activity on *Colletotrichum acutatum*

M. ovata essential oils showed differentiated levels of toxicity on *C. acutatum*. The nerolic acid + linalool chemotype showed lower MIC (0.03 $\mu L.mL^{-1}$), followed by the nerolic acid, geraniol, and isopulegol + linalool chemotypes, with MICs of 0.05, 0.08, and 0.09 $\mu L.mL^{-1}$, respectively (Table 3).

The major compounds nerolic acid and nerolic acid + linalool presented a lower MIC (0.06 $\mu L.mL^{-1}$), followed by geraniol, with an MIC of 0.08 $\mu L.mL^{-1}$. The pure compound citral had an MIC of 0.1 $\mu L.mL^{-1}$, and the compounds (*E*)-nerolidol and linalool had the highest MIC values (0.5 and 0.9 $\mu L.mL^{-1}$), leading to lower toxicity against *C. acutatum* among all the treatments (Table 3). The chemotype and major compound citral + (*E*)-nerolidol showed the same value of MIC and MFC (0.2 $\mu L.mL^{-1}$) (Table 3 and Table 4).

Regarding the MFC, the major compounds nerolic acid and nerolic acid + linalool presented greater fungicidal activity than their essential oils. The major compounds geraniol and citral + (*E*)-nerolidol had the same result. The major compound nerolic acid + linalool presented the lowest MFC (0.1 $\mu L.mL^{-1}$) against *C. acutatum* (Table 4).

The geraniol and citral + (*E*)-nerolidol chemotypes, their major compounds, and the pure compound citral showed an MFC of 0.2 $\mu L.mL^{-1}$. The major compound nerolic acid presented an MFC of 0.5 $\mu L.mL^{-1}$, and the nerolic acid and nerolic acid + linalool chemotypes had an MFC of 0.7 $\mu L.mL^{-1}$. Finally, the isopulegol + linalool chemotype and the pure compounds linalool and (*E*)-nerolidol presented an MFC of 1.0 $\mu L.mL^{-1}$ (Table 4).

In vitro antifungal activity on *Plenodomus destruens*

The phytopathogen *P. destruens* was more sensitive to the nerolic acid chemotype that presented the lowest MIC (0.05 $\mu L.mL^{-1}$), followed by nerolic acid + linalool chemotype with 0.06 $\mu L.mL^{-1}$. Geraniol chemotype and the major compounds geraniol, nerolic acid, nerolic acid + linalool, and citral + (*E*)-nerolidol showed an MIC of 0.07 $\mu L.mL^{-1}$ (Table 5).

The pure compound citral and the citral + (*E*)-nerolidol chemotype showed the same value of MIC (0.1 $\mu L.mL^{-1}$), followed by isopulegol + linalool and (*E*)-nerolidol, with an MIC of 0.5 $\mu L.mL^{-1}$. Linalool presented the highest MIC (1.0 $\mu L.mL^{-1}$), being the least toxic treatment against *P. destruens* (Table 5).

For MFC, the major compounds nerolic acid and geraniol had more antifungal properties than their respective essential oil chemotypes; however, for the major compounds nerolic acid + linalool and citral + (*E*)-nerolidol, the result was the same. The nerolic acid + linalool chemotype and the major compound presented the same MFC (0.07 $\mu L.mL^{-1}$) against *P. destruens*, followed by geraniol chemotype and the major compound citral + (*E*)-nerolidol, with an MFC of 0.1 $\mu L.mL^{-1}$ (Table 6).

The pure and major compounds citral and geraniol showed an MFC of 0.2 $\mu L.mL^{-1}$, followed by the nerolic acid chemotype, with an MFC of 0.3 $\mu L.mL^{-1}$. The pure and major compounds (*E*)-nerolidol and nerolic acid presented an MFC of 0.5 $\mu L.mL^{-1}$, and the isopulegol + linalool chemotype showed the highest MFC (0.6 $\mu L.mL^{-1}$). Linalool was less toxic to *P. destruens* (Table 6).

In vitro antifungal properties on *Thielaviopsis paradoxa*

Among all the treatments tested on *T. paradoxa*, the major compound citral + (*E*)-nerolidol presented the lowest MIC (0.02 $\mu L.mL^{-1}$). Nerolic acid, nerolic acid + linalool, geraniol, citral + (*E*)-nerolidol chemotypes; the major compounds nerolic acid + linalool and geraniol; and the pure compound citral showed an MIC of 0.04 $\mu L.mL^{-1}$ (Table 7).

The major compound nerolic acid, the isopulegol + linalool chemotype, and the pure compound linalool presented MICs of 0.05, 0.1, and 0.2 $\mu L.mL^{-1}$, respectively. No MIC result was detected for the pure compound (*E*)-nerolidol among the tested concentrations (Table 7).

Concerning the MFC, the major compounds nerolic acid, nerolic acid + linalool, and geraniol presented the highest fungicidal properties when compared with their respective essential oil chemotypes. The chemotype and major compound citral + (*E*)-nerolidol presented the same results (Table 8).

The citral + (*E*)-nerolidol chemotype, the major compounds citral + (*E*)-nerolidol and geraniol, and the pure compound citral presented a lower MFC (0.2 $\mu L.mL^{-1}$), followed by the geraniol chemotype and the major compounds nerolic acid + linalool and nerolic acid, with an MFC of 0.5, 0.6, and 0.8 $\mu L.mL^{-1}$, respectively. For the other treatments, no MFC was detected among the tested concentrations (Table 8).

Table 3. Percentage of *in vitro* mycelial growth inhibition of *Colletotrichum acutatum*, according to different concentrations of essential oils of *Myrcia ovata* chemotypes and major and pure compounds, after nine days of incubation.

	Individuals (chemotypes)					Major compounds			Pure compounds			
Concentration ($\mu L.mL^{-1}$)	MYRO-159 (Nerolic acid)	MYRO-180 (Nerolic acid + linalool)	MYRO-388 (Geraniol)	MYRO-157 (Citral + (*E*)-nerolidol)	MYRO-174 (Isopulegol + linalool)	Nerolic acid	Nerolic acid + linalool	Geraniol	Citral + (*E*)-nerolidol	Citral	(*E*)-nerolidol	Linalool
0.01	49.8 ± 0.1	46.1 ± 0.2	16.1 ± 0.2	21.3 ± 0.3	26.9 ± 0.4	36.8 ± 0.0	13.0 ± 0.3	23.3 ± 0.2	14.8 ± 0.2	21.7 ± 0.4	11.5 ± 0.2	12.8 ± 0.3
0.02	71.7 ± 0.1	83.9 ± 0.3										
0.03	85.7 ± 0.1	100 ± 0.0	49.4 ± 0.4	39.8 ± 0.3	50.2 ± 0.2	69.3 ± 0.1	33.7 ± 0.4	57.6 ± 0.2	43.1 ± 0.1	23.5 ± 0.2	18.5 ± 0.1	
0.05	100 ± 0.0		75.7 ± 0.1	46.4 ± 0.2	79.6 ± 0.1	87.1 ± 0.1	77.0 ± 0.1	80.6 ± 0.1		31.9 ± 0.3	26.5 ± 0.2	
0.06						100 ± 0.0	100 ± 0.0					
0.07			88.9 ± 0.0	77.2 ± 0.2				86.7 ± 0.1	74.1 ± 0.1	53.0 ± 0.2	38.7 ± 0.0	
0.08			100 ± 0.0		90.0 ± 0.0			100 ± 0.0				
0.09					100 ± 0.0					90.0 ± 0.0		
0.1										100 ± 0.0	56.5 ± 0.1	17.4 ± 0.1
0.2				100 ± 0.0					100 ± 0.0			
0.4											60.7 ± 0.1	
0.5											100 ± 0.0	40.9 ± 0.3
0.8												90.0 ± 0.0
0.9												100 ± 0.0

Table 4. Fungicidal activity of essential oils of *Myrcia ovata* chemotypes and major and pure compounds on *in vitro* mycelial growth inhibition of *Colletotrichum acutatum* after 96 hours of incubation.

	Individuals (chemotypes)					Major compounds			Pure compounds			
Concentration ($\mu L.mL^{-1}$)	MYRO-159 (Nerolic acid)	MYRO-180 (Nerolic acid + linalool)	MYRO-388 (Geraniol)	MYRO-157 (Citral + (*E*)-nerolidol)	MYRO-174 (Isopulegol + linalool)	Nerolic acid	Nerolic acid + linalool	Geraniol	Citral + (*E*)-nerolidol	Citral	(*E*)-nerolidol	Linalool
0.1							100 ± 0.0					
0.2			100 ± 0.0	100 ± 0.0				100 ± 0.0	100 ± 0.0	100 ± 0.0		
0.5						100 ± 0.0						
0.7	100 ± 0.0	100 ± 0.0										
1.0					100 ± 0.0						100 ± 0.0	100 ± 0.0

Table 5. Percentage of *in vitro* mycelial growth inhibition of *Plenodomus destruens*, according to different concentrations of essential oils of *Myrcia ovata* chemotypes and major and pure compounds, after seven days of incubation.

	Individuals (chemotypes)					Major compounds			Pure compounds			
Concentration ($\mu L.mL^{-1}$)	MYRO-159 (Nerolic acid)	MYRO-180 (Nerolic acid + linalool)	MYRO-388 (Geraniol)	MYRO-157 (Citral + (*E*)-nerolidol)	MYRO-174 (Isopulegol + linalool)	Nerolic acid	Nerolic acid + Geraniol linalool		Citral + (*E*)-nerolidol	Citral	(*E*)-nerolidol	Linalool
0.01	53.0 ± 0.1	52.6 ± 0.2	0.0 ± 0.0	29.4 ± 0.1	0.0 ± 0.0	33.1 ± 0.3	28.2 ± 0.2	28.2 ± 0.2	27.8 ± 0.1	10.0 ± 0.0	20.0 ± 0.3	5.6 ± 0.0
0.02	60.0 ± 0.2	61.9 ± 0.1										
0.03	91.1 ± 0.0		9.8 ± 0.4	35.2 ± 0.2	1.9 ± 0.1	63.1 ± 0.1	54.8 ± 0.2	54.8 ± 0.2	55.9 ± 0.3	13.6 ± 0.1	27.9 ± 0.3	8.1 ± 0.1
0.04			46.7 ± 0.3									
0.05	100 ± 0.0	81.5 ± 0.2				76.9 ± 0.2	83.2 ± 0.2	81.0 ± 0.1	60.9 ± 0.1	28.7 ± 0.4	44.8 ± 0.0	14.4 ± 0.1
0.06		100 ± 0.0	64.4 ± 0.2	47.5 ± 0.1								
0.07			100 ± 0.0		31.9 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0			
0.1					50.9 ± 0.5						69.6 ± 0.1	
0.2				100 ± 0.0						100 ± 0.0		
0.5					100 ± 0.0						100 ± 0.0	36.9 ± 0.2
1.0												100 ± 0.0

Table 6. Fungicidal properties of essential oils of *Myrcia ovata* chemotypes and major and pure compounds on *in vitro* mycelial growth inhibition of *Plenodomus destruens* after 96 hours of incubation.

	Individuals (chemotypes)					Major compounds			Pure compounds			
Concentration ($\mu L.mL^{-1}$)	MYRO-159 (Nerolic acid)	MYRO-180 (Nerolic acid linalool)	MYRO-388 + (Geraniol)	MYRO-157 (Citral + (*E*)-nerolidol)	MYRO-174 (Isopulegol + linalool)	Nerolic acid	Nerolic acid linalool	+ Geraniol	Citral + (*E*)-nerolidol	Citral	(*E*)-nerolidol	Linalool
0.07		100 ± 0.0					100 ± 0.0					
0.1			100 ± 0.0						100 ± 0.0			
0.2				100 ± 0.0				100 ± 0.0		100 ± 0.0		
0.3	100 ± 0.0											
0.5						100 ± 0.0					100 ± 0.0	
0.6					100 ± 0.0							

DISCUSSION

All treatments presented antifungal properties, showing different levels of *in vitro* toxicity among the tested fungi. In relation to the fungus *C. acutatum*, the essential oils of MYRO-159 and MYRO-180 (nerolic acid and nerolic acid + linalool chemotypes) presented a higher MIC than their respective major compounds. Conversely, the major compounds geraniol and citral + (*E*)-nerolidol presented the same MIC as the chemotypes.

Regarding the MFC, the same treatments presented low and equal results. Also, when comparing the properties of the pure compounds citral, (*E*)-nerolidol, and linalool with their respective mixtures, linalool and (*E*)-nerolidol showed lower toxicity when used as pure compounds. These results suggest the existence of some compound(s) at a lower percentage in the essential oils (minor compounds), which contribute to their properties, resulting, therefore, in a synergic effect. Conversely, the results of citral infer the presence of an antagonistic effect.

For the fungus *P. destruens*, the essential oils of MYRO-159 and MYRO-180, of nerolic acid and nerolic acid + linalool chemotypes, showed a higher MIC than their respective major compounds. The essential oil of MYRO-388, of the geraniol chemotype, and its major compound presented the same results, and the essential oil of MYRO-157, of the citral + (*E*)-nerolidol chemotype, presented lower properties than its major compound.

For the MFC, the essential oils of MYRO-159 and MYRO-388, of the nerolic acid and geraniol chemotypes, presented higher properties than their major compounds. Equal results were obtained for the essential oil of MYRO-180, of the nerolic acid + linalool chemotype, and its major compound. The essential oil of MYRO-157, of the citral + (*E*)-nerolidol chemotype, showed weaker properties than its major compound. The pure compounds citral, (*E*)-nerolidol, and linalool demonstrated lower toxicity when compared with their mixtures, leading to a synergic effect of the minor compounds present in the essential oils of MYRO-159, MYRO-180, MYRO-157, and MYRO-174.

For the fungus *T. paradoxa*, only the essential oil of MYRO-159, of the nerolic acid chemotype, presented higher MIC than its major compound. The essential oils of MYRO-180 and MYRO-157, of the nerolic acid + linalool and geraniol chemotypes, presented same results to those of their major compounds, and the essential oil of MYRO-157, of the citral + (*E*)-nerolidol chemotype, had lower MIC than its major compound.

In relation to the MFC, the essential oils of MYRO-159 and MYRO-180, of the nerolic acid and nerolic acid + linalool chemotypes, presented higher properties than their major compounds. The essential oil of MYRO-157, of the citral + (*E*)-nerolidol chemotype, had the same result as its major compound, and the essential oil of MYRO-388, of the geraniol chemotype, showed weaker properties than its major compound. When comparing the pure compound citral with its respective mixtures, all treatments showed the same results, implying that the minor compounds do not interfere with citral properties, resulting in an indifferent effect on *T. paradoxa* MFC.

It is well known that the presence of other compounds at lower concentrations may increase or decrease the antifungal effect of the essential oil (NIZIO et al., 2015). Despite the poor characterization of the mechanisms of essential oils action, they are involved in the alteration of the cell membrane permeability, causing the leakage of its contents (PIPER et al., 2001); the inhibition of protein and enzymes synthesis linked to cellular respiration due to lipophilic compounds; and the alteration of the proton motor forces owing to variations in pH and electric potential (FRAZÃO et al., 2017).

Essential oils and their major compounds were tested against *C. acutatum* in an experiment that evaluated strawberry anthracnose. When using linalool enantiomers, in bioautography tests, an inhibitory action was detected in a four-day period at a concentration of 20 $mg.mL^{-1}$ (ÖZEK et al., 2010). Thymol and carvacrol compounds presented 100% mycelial growth inhibition in a 240-hour period at a concentration of 150 $\mu g.mL^{-1}$ (NUMPAQUE et al., 2011). The essential oils of *Thymus zygis* (spp. *sylvestris* and spp. *gracilis*) (Lamiaceae), obtained at the flowering stage, showed antifungal activity on *C. acutatum*, with a mean Effective Concentration (EC_{50}) of 0.11 and 0.18. Moreover, the Spearman's correlation of the essential oil of *T. zygis* revealed that the minor compounds citral (0.1%), β-pinene (0.2%), and limonene (0.3%) presented higher correlation effect than the major compounds *p*-cymene (16,8%) and thymol (63.4%), concluding that the properties of this essential oil is a result of the minor compounds (PÉREZ-SÁNCHEZ et al., 2007).

Table 7. Percentage of *in vitro* mycelial growth inhibition of *Thielaviopsis paradoxa*, according to different concentrations of essential oils *Myrcia ovata* chemotypes and major and pure compounds, after 54 hours of incubation.

	Individuals (chemotypes)				Major compounds				Pure compounds			
Concentration ($\mu L.mL^{-1}$)	MYRO-159 (Nerolic acid)	MYRO-180 (Nerolic acid + linalool)	MYRO-388 (Geraniol)	MYRO-157 (Citral + (*E*)-nerolidol)	MYRO-174 (Isopulegol + linalool)	Nerolic acid	Nerolic acid + Geraniol linalool		Citral + (*E*)-nerolidol	Citral	(*E*)-nerolidol	Linalool
0.01	70.0 ± 0.1	52.0 ± 0.2	38.9 ± 0.1	50.9 ± 0.2	16.5 ± 0.2	52.0 ± 0.1	61.1 ± 0.2	25.9 ± 0.1	64.8 ± 0.1	26.5 ± 0.3	2.8 ± 0.3	59.1 ± 0.1
0.02	77.5 ± 0.0	73.1 ± 0.1	69.1 ± .03	70.0 ± 0.1			66.5 ± 0.5	54.7 ± 0.1	100 + 0.0	50.4 ± 0.2		
0.03	90.0 ± 0.0	90.0 ± 0.0	74.2 ± 0.1	90.9 ± 0.0	47.2 ± 0.1	87.8 ± 0.0	79.3 ± 0.3	79.1 ± 0.1		72.0 ± 0.1		67.2 ± 0.0
0.04	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0		90.9 ± 0.0	100 + 0.0	100 ± 0.0		100 ± 0.0		
0.05					63.3 ± 0.1	100 ± 0.0					12.6 ± 0.1	74.2 ± 0.1
0.1					100 ± 0.0						16.7 ± 0.2	84.3 ± 0.1
0.2												100 + 0.0
1.0											66.4 ± 0.0	

Table 8. Fungicidal properties of essential oils of *Myrcia ovata* chemotypes and major and pure compounds on *in vitro* mycelial growth inhibition of *Thielaviopsis paradoxa* after 96 hours of incubation.

	Individuals (chemotypes)					Major compounds			Pure compounds			
Concentration ($\mu L.mL^{-1}$)	MYRO-159 (Nerolic acid)	MYRO-180 (Nerolic acid + linalool)	MYRO-388 (Geraniol)	MYRO-157 (Citral + (*E*)-nerolidol)	MYRO-174 (Isopulegol + linalool)	Nerolic acid	Nerolic acid + Geraniol linalool		Citral + (*E*)-nerolidol	Citral	(*E*)-nerolidol	Linalool
0.2				100 ± 0.0				100 ± 0.0	100 ± 0.0	100 ± 0.0		
0.5			100 ± 0.0									
0.6							100 ± 0.0					
0.8						100 ± 0.0						
1.0												

No scientific papers have addressed the antifungal properties of essential oils and compounds against *P. destruens*. Geraniol isolated from the essential oil of *Zanthoxylum alatum* (Rutaceae) fruits presented antifungal properties against *Ceratocystis paradoxa* (the sexual form of *T. paradoxa*) that attacked sugarcane crops (RAO & SINGH, 1994).

The essential oils of *Lippia sidoides* (Verbenaceae) and its major compounds showed antifungal activity against *T. paradoxa*. A 100% mycelial growth inhibition was detected at concentrations of 0.2 and 0.3 $\mu L.mL^{-1}$ (MIC) for the essential oil and major compound thymol, respectively, highlighting the synergic effect of minor compounds on the major compound thymol (CARVALHO et al., 2013). These authors also concluded that even though the pure compounds carvacrol, 1,8-cineole, α-terpinene, β-caryophyllene, and p-cymene had no direct influence on the mycelial growth of *T. paradoxa* at concentrations below or equal to 0.5 $\mu L.mL^{-1}$, these compounds (or other minor compounds) present in the essential oil act synergistically against the fungus.

In conclusion, for *C. acutatum*, the essential oil from the individual MYRO-180, of the nerolic acid + linalool chemotype, and its major compound showed the lowest MIC and MFC (0.03 and 0.1 $\mu L.mL^{-1}$, respectively). For *P. destruens,* the essential oil from the individual MYRO-159, of the nerolic acid chemotype, presented the lowest MIC (0.05 $\mu L.mL^{-1}$). Finally, the nerolic acid + linalool chemotype and the major compound both presented an MFC of 0.07 $\mu L.mL^{-1}$. For *T. paradoxa,* the major compound citral + (*E*)-nerolidol stood out with the lowest MIC and MFC (0.03 and 0.2 $\mu L.mL^{-1}$, respectively). Linalool showed the lowest toxicity against the three tested fungi.

These results present low values of MIC for 100% *in vitro* mycelial growth inhibition, emphasizing the importance of this work. Nevertheless, future studies on *in vivo* evaluations are recommended since the effects of *in vitro* evaluations usually have low MIC values (FRAZÃO et al., 2017).

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RESUMO: *Myrcia ovata,* uma espécie nativa de fitofisionomia de Restinga, possui atividade antifúngica. Os fitopatógenos *Colletotrichum acutatum, Plenodomus destruens* e *Thielaviopsis paradoxa* são responsáveis pelas doenças podridão floral de citros, mal-do-pé da batata doce e resinose do coqueiro, respectivamente. A atividade antifúngica de cinco quimiotipos de *M. ovata* (MYRO-159, quimiotipo ácido nerólico; MYRO-180, ácido nerólico + linalol; MYRO-388, quimiotipo geraniol; MYRO-157, quimiotipo citral + (E)-nerolidol; e, MYRO-174, quimiotipo isopulegol + linalol), quatro compostos majoritários (ácido nerólico, ácido nerólico + linalol, geraniol e citral + (E)-nerolidol) e três compostos isolados (citral, (E)-nerolidol e linalol) foram avaliados sobre os fungos *C. acutatum*, *P. destruens* e *T. paradoxa*. Testes *in vitro* foram conduzidos em delineamento inteiramente casualizado com três repetições e concentrações (*v*/*v*), que variaram de 0,01 a 1,0 $\mu L.mL^{-1}$. Todos os tratamentos testados apresentaram atividade antifúngica. Para o fungo *C. acutatum*, o óleo essencial do indivíduo MYRO-180, de quimiotipo ácido nerólico + linalol, e seu composto majoritário apresentaram menores Concentração Mínima Inibitória (CMI) e Concentração Mínima Fungicida (CMF) de 0,03 e 0,1 $\mu L.mL^{-1}$, respectivamente. Para o fungo *P. destruens*, o óleo essencial do indivíduo MYRO-159, de quimiotipo ácido nerólico, apresentou menor CMI de 0,05 $\mu L.mL^{-1}$, e o quimiotipo ácido nerólico + linalol e seu composto majoritário apresentaram a menor CMF de 0,07 $\mu L.mL^{-1}$. Para o fungo *T. paradoxa*, a combinação de citral + (E)-nerolidol destacou-se com CMI e CMF de 0,03 e 0,2 $\mu L.mL^{-1}$, respectivamente. Linalol foi o menos tóxico sobre os três fungos testados.

PALAVRAS-CHAVE: Myrtaceae. *Colletotrichum acutatum*. *Plenodomus destruens*. *Thielaviopsis paradoxa*.

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