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Chrysopogon zizanioides (L.) Roberty essential oil as *Pinus* wood resistance enhancer against the action of xylophagous fungus *Rhodonia placenta*

Henrique Trevisan[1]✤, Vinicius José Fernandes[1], Claudio Eduardo Rodrigues-Santos[2]

[1]Federal Rural University of Rio de Janeiro, Institute of Forestry, Wood Deterioration Laboratory, Seropédica, Rio de Janeiro, Brazil.
[2]Federal Rural University of Rio de Janeiro, Institute of Chemistry, Laboratory of Synthesis and Medicinal Chemistry, Seropédica, Rio de Janeiro, Brazil.

TECHNOLOGY OF FOREST PRODUCTS

ABSTRACT

Background: Plant essential oils have been tested as strategy to increase wood durability and to optimize its use. The aim of the present study is to assess the efficiency of *Chrysopogon zizanioides* essential oil produced in Brazil and in China to increase *Pinus* wood resistance against the wood-destroying fungus *Rhodonia placenta*. Wood specimens were exposed to fungus *Rhodonia placenta* and treated with these oils at concentrations 10%, 25%, 50% and 100%. Gas chromatography in combination to mass spectrometer was performed to feature the oil components.

Results: The Brazilian oil turned *Pinus* wood highly resistant to *R. placenta* and the Chinese one made it partially resistant to this fungus. Brazilian sample presented compounds typical of *C. zizanioides,* namely: khusimol, β-vetivone, vetiselinenol and β-vetisperene. The Chinese oil only presented few sesquiterpenes, among them, *cis*-thujopsene, cedrol and pachoulol. Isopropyl myristate was its major component, but it does not have plant origin.

Conclusion: Therefore, *Pinus* wood is highly susceptible to fungus *R. placenta,* but it gets resistant to it after being treated with typical *C. zizanioides* essential oil at concentrations of 25%, 50% and 100%. *C. zizanioides* essential oil added with additives, such as isopropyl myristate, loses efficiency in making *Pinus* wood resistant to fungus *R. placenta*.

Keywords: Wood protection; wood deterioration; xylophagous organisms; natural products.

HIGHLIGHTS

First report on *C. zizanioides* essential oil into *Pinus* wood for protection against xylophagous fungi.
The oil of *C. zizanoides* adds resistance to pine wood against the fungus *R. placenta*.
Brazilian *C. zizanioides* oil contains typical chemical markers in its composition.
The essential oil of *C. zizanoides* shows potential for treating wood against deterioration.

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✤Corresponding author: hentrevisan@gmail.com
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INTRODUCTION

Wood is abundant in nature, besides coming from renewable sources. It is very important for construction projects, as well as for furniture and boat manufacturing. Despite these relevant features, it is a natural polymer mainly made out of cellulose, lignin and hemicellulose. These components turn wood into food source for several wood-eating organisms, including fungi (Mariano et al., 2020). These features hinder its proper use, mainly that of wood from forest species susceptible to these organisms, such as *Pinus* sp. (Martin and Lopez, 2023).

The aforementioned problem and the search for optimizing this material's use demands the application of methods aimed at increasing wood durability by improving its resistance to these organisms' action (Cruz-Lopes et al., 2025; Trevisan et al., 2020). Wood chemical treatment is among these methods; furthermore, it has been the main method adopted by the industrial sector (Lepage et al., 2017). However, several questions, mainly those about the environmental safety of used inputs, point towards the need of research aimed at developing alternative methods to increase wood resistance. This research has been implemented, and it also seeks to reduce environmental risks (Cruz-Lopes et al., 2025; Santos et al., 2022).

Assessing plants' natural substances is among the focuses of such studies and it has been a promising path to protect wood from deterioration (Wozniak, 2022). Brada (2022) pointed out several studies, according to which, these substances are capable of protecting wood against the action of xylophagous fungi. Some of these substances are found in essential oils.

Essential oils are natural, volatile and aromatic substances extracted from plants. They are non-greasy, partially soluble in water, flammable, soluble in alcohol and easily absorbed by the human body. Many of them have biological activities such as antioxidant, bactericidal, fungicidal and insecticidal functions. They comprise a whole variety of substances, including aromatic hydrocarbons, aliphatic sesquiterpenes, terpenes and oxygenated derivatives (Sousa, 2024).

Poacea Chrysopogon zizanioides (L.) Roberty is an Asian grass species native to India. Its roots' essential oil has complex chemical composition. It is widely used by the pharmaceutical and cosmetic industries given its several biological properties, including fungicide, insecticide and antioxidant (David et al., 2023; Soidrou et al., 2013). The vetiver essential oil obtained from vetiver root is a viscous oil with brown color having a green-woody, earthy fragrance (Verma, 2019; Pfaff et al., 2019). Soidrou et al. (2013) observed that this oil inhibits the development of four wood-eating fungi species in culture medium, even at low concentrations. However, although the results presented by Soidrou et al. (2013) were promising *in vitro*, they emphasized the need for further studies. Thus, and by bearing in mind that Pinus wood is known for its low durability, although being extremely important as timber source, it is paramount assessing *C. zizanioides* essential oil ability to add fungal resistance to it. Among the decay fungi, *Rhodonia placenta* causes brown rot, that often have high deterioration ability. These fungi prefer cellulose and hemicellulose, and they do not affect lignin. Wood attacked by these fungi becomes dry, powdery, as well as presents dark brown color and acquires burnt and cracked appearance (Mariano et al., 2020).

From this perspective, the aim of the present study is to assess the efficiency of treating *Pinus* wood with *C. zizanioides* essential oil produced in China and in Brazil to make it resistant to the action of xylophagous fungus *R. placenta*.

MATERIALS AND METHODS

The *Pinus* wood came from two felling trees grown at the Federal Rural University of Rio de Janeiro (UFRRJ) campus. Wood boards and battens were cut from the trunk, from the log's external region. They were used to get 90 test specimens (2.5 x 2.5 x 0.9 cm) as recommended by AWPA E10-16.

C. zizanioides essential oil was purchased from two suppliers, one from Brazil and another from China. Both the Brazilian and the Chinese oils were pure and manufactured through *C. zizanioides* root hydrodistillation. Five concentrations of each oil were used to treat the samples, namely: 10%, 25%, 50% and 100%. Dilutions were made in 95% P.A. (ethyl alcohol diluent) and manipulation was carried out in horizontal laminar flow hood. The diluted solutions were stored in sterilized glass vials (10 mL) sealed with stopper and cap.

All wood samples were acclimatized to 20 ± 2 ºC and 65 ± 5 %, reaching 13% moisture content. Ten specimens per concentration were treated by homogeneously brushing the whole surface of the samples, on two coats, after the respective solutions were prepared. Subsequently, the samples were left to rest inside the horizontal laminar flow hood for 30 minutes, under ultraviolet light, to reduce the possibility of contamination.

AWPA E10-16 standard guidelines were used to conduct the trial with fungus *R. Placenta*. In total, 45 glass bottles (500 ml) with screw caps were used; they were filled with 180 g soil and 40 ml distilled water. These bottles received two *Pinus* slides (3 cm x 3 cm) each and were sterilized in laboratory autoclave. Then, the *Pinus* slides were inoculated with fungus *R. Placenta* coming from pure cultures kept in PDA culture medium. This procedure was performed in horizontal laminar flow hood.

The treated specimens sterilized under ultraviolet light were placed under these slides, two per flask, after the fungus had developed on these slides for 30 days. This step was also performed in laminar flow hood. The samples remained under the action of *R. placenta* for four months. Then, they were cleaned and acclimated at 70°C until reaching constant weight in order to be weighed again. Mass loss percentage caused by the action of the fungus was calculated by subtracting the value measured before samples' subjection to *R. placenta* from that measured after they were subjected to it. These values were used to classify wood into strength classes (Table 1).

Table 1: Wood resistance classification criteria based on mass loss caused by the action of fungus *R. placenta* (AWPA E30-16, 2016).

Average Weight Loss (%)	Class Of Resistance
0 a 10	Highly resistant
11 a 24	Resistant
25 a 44	Moderately resistant
45 ou acima	Not resistant or perishable

Data analysis was performed in BioEstat 5.0 software (Ayres et al., 2007). Normality was assessed through Lilliefors test (5% significance). Non-parametric Kruskal-Wallis test with Dunn's post-test (5% significance) was used to analyze mean ranks' variances due to the recorded non-normal distribution.

Identification of Compounds

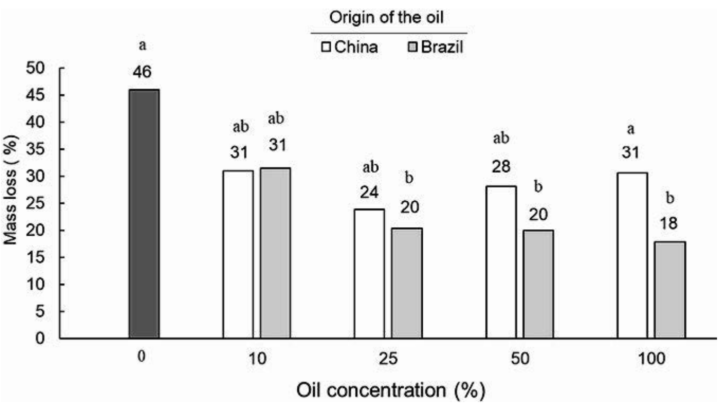
The samples were prepared by diluting each volatile oil in diethyl ether at concentration of 10 mg/ml. Then, 1 µL of each sample was injected into a gas chromatograph (5890 Series II, Hewlett-Packard, USA) equipped with a flame ionization detector (GC-FID) operating in split mode (1:20). It was done to separate and quantify the constituents in the volatile oils. The observed compounds were separated in a fused silica capillary column at stationary phase comprising 5% phenyl and 95% dimethylpolysiloxane (30 m × 0.25 mm × 0.25 µm ID). Helium was the carrier gas; it was used at flow rate of 1 ml/min. Column temperature program was 40 °C for 25 min followed by increase from 2 °C/min to 250 °C, which was held for 20 min. Injector and detector temperatures were 250 °C and 290 °C, respectively.

The same sample and volume (1 µL) were injected into a gas chromatograph coupled to a mass spectrometer (GC-MS) QP-2010 Plus (Shimadzu, Japan) n order to separate and identify the volatile oil compounds. Both the column and the software were the same used for the GC-FID analysis. Injector and interface temperatures were 220 °C and 250 °C, respectively. Mass spectrum was found in quadrupole detector operating at 70 eV, with mass ranging from 40 to 4100 m/z and scan rate of 0.5 scans/second. Compounds found in the volatile oil were quantified based on GC-FID peak areas and converted into percentage values. Compound identification in the volatile oil was based on GC-MS analysis, and it took into consideration the retention index (RI) calculation. This index, in its turn, was based on a homologous series of n-alkanes (C7-C30) that were injected under the same conditions applied to the sample. Data were processed in GC-MS Solution software, v.2.53 (Shimadzu). Each compound in the volatile oil sample was identified by comparing the mass spectra to a database (NIST23s.lib) and to the literature (Adams, 2007).

RESULTS

Pinus wood treatment with *C. zizanioides* essential oil produced in China and in Brazil reduced the wood's mass loss caused by the action of xylophagous fungus *R. placenta*. However, this oils' reduction efficiency changed depending on its origin and concentration. Therefore, all samples treated with the Chinese oil, at any adopted concentration, presented mass loss reduction statistically equivalent to that of fresh wood. On the other hand, samples treated with the Brazilian oil only recorded mass loss statistically equivalent to that of fresh wood at the concentration of 10%. The other treatments based on Brazilian oil at concentrations of 25%, 50% and 100% recorded significant reduction in comparison to the control (Figure 1).

Fresh *Pinus* wood was classified as non-resistant to the action of *R. placenta*. However, it was classified as moderately resistant when it was treated with the Chinese *C. zizanioides* essential oil under all tested conditions; the same outcome was only observed for the treatment with Brazilian oil at concentration of 10%. The remarkable resistance to the fungus' action was observed for samples subjected to treatment with Brazilian oil at concentrations of 25%, 50% and 100%, which allowed classifying the *Pinus* wood as resistant to *R. placenta* (Table 2).



Different letters, between bars, express statistically significant differences (Dunn, 5% significance level).

Figure 1: Mean mass loss (%) of *Pinus sp.* wood treated with *Chrysopogon zizanioides* oil produced in China and in Brazil, at four concentrations, subjected to the action of xylophagous fungus *Rhodonia placenta*, for four months, under laboratory conditions.

Table 2: Mean wood mass loss percentage and strength class of *Pinus sp.* wood treated with *Chrysopogon zizanioides oil* produced in China and in Brazil, at four concentrations and subjected to the action of xylophagous fungus *Rhodonia placenta*, for four months.

Concentration (%)	China		Brazil	
	Mass loss (%)	Class Of Resistance	Mass loss (%)	Class Of Resistance
0	46	Not resistant	46	Not resistant
10	31	Moderately resistant	31	Moderately Resistant
25	24	Moderately resistant	20	Resistant
50	28	Moderately resistant	20	Resistant
100	31	Moderately resistant	18	Resistant

The GC-MS analysis applied to the Brazilian and Chinese oils showed different compositions between major components (Tables 3 and 4).

The Brazilian oil chemical composition shows the typical presence of oil extracted from *C. zizanioides*. Its main markers are khusimol (18.8%), β-vetivone (5.1%), vetiselinenol (6.4%) and β-vetisperene (0.4%). The major components found at concentrations higher than 5% were khusimol (18.8%), α-vetivone (10.7%), nootkatone (6.6%), vetiselinenol (6.4%), cubenol (6.4%) and β-vetivone (5.2%) (Figure 2).

The following elements stood out in the hydrocarbon fraction of the Brazilian sample: α-muurolene (2.3%), khusimene (0.6%), β-vetisperene (0.4%) and prezizaene (0.4%). The extract presented one fraction rich in alcohols, namely: khusimol (18.8%), vetiselinenol (6.4%), cubenol (6.6%), E-isovalencenol (2.7%), selin-6-en-4α-ol (2.6%), junenol (2.0%), β-eudesmol (1.3%), zizanol (1.3%) and alocedrol (3.4%). Ketones such as α-vetivone (10.7%), nootkatone (6.6%), β-vetivone (5.2%), khusimone (1.9%) and epizyzanone (1.9%) were also identified among the oxygenate compounds.

The composition of the Chinese oil mainly counted on hydrocarbons. The major components identified in it were isopropyl myristate (38.8%), cis-thujopsene (9.8%), β-funebrene (3.6%), 2-methyltetradecane (1.7%), cedrol (4.5%) and pachoulol (2.7%).

DISCUSSION

C. zizanioides essential oil fungicidal property against fungus *R. placenta in vitro* was proven by Soidrou et al. (2013), according to whom, concentrations of 1%, 0.4%, 0.2%, 0.1%, 0.05%, 0.03% and 0.02% fully inhibited the fungus' development. Based on this relevant outcome, Soidrou et al. (2013) raised the hypothesis that this oil can provide fungal resistance when it is incorporated right into the wood, and their finding was corroborated by *Pinus* sp. wood's behavior under the action of *R. placenta* observed in the current study.

Results have shown that this fungal resistance's contribution was less significant when the Chinese oil was applied to the wood in comparison to the treatment based on the application of the Brazilian oil. Therefore, it is worth clarifying that, although the wood treatment with Chinese oil did not lead to significant reduction in mass loss in comparison to the control, it is worth noticing that this treatment allowed classifying the wood as partially resistant to *R. placenta*, according to criteria by AWPA E30-16, 2016. The fresh wood and that treated with the Brazilian oil were classified as non-resistant and resistant, respectively. Therefore, both the Chinese and the Brazilian oils gave fungal resistance to the wood, but at different efficiency rates. This finding can be justified by these oils' different chemical compositions.

Reports, such as those by Dubey et al. (2010), have shown the greater efficacy of the *C. zinanoides* oil produced in Southern India against fungus *Rhizoctonia solani* in comparison to oil produced in the Northern region of this country. This outcome evidences that variations in antifungal activity can be influenced by the plants' geographical origin. The different chemical profile shown by this oil, when it is produced in different regions, was presented by Pandey e Tiwari (2024). Their finding corroborates information provided earlier in this section. They reported that samples from China, Turkey, Comoros, Brazil, India, Thailand, Taiwan, Egypt and Indonesia presented the following amount of compounds: 11, 26, 34, 13, 18, 35, 23, 36 and 37, respectively. According to them, this difference results from factors such as climate, soil, altitude and *C. zizanioides* irrigation conditions, cultivation techniques and harvest time, as well as oil extraction method. All these variables can significantly change plants' chemical profile.

Therefore, reports by Dubey et al. (2010) and Pandey e Tiwari (2024) allow assuming that plants' geographical origin could have had negative influence on the efficiency of the Chinese oil in inhibiting fungus *R. placenta*. Nevertheless, although both oils were pure and extracted through *C. zizanioides* roots' hydrodistillation, the chromatographic analysis pointed towards the hypothesis that chemical composition differences derived from the manufacturing process rather than from the different geographical origin of plants used as raw material. This understanding is mainly supported by the isopropyl myristate (38.8%) mainly found in the Chinese oil, which is an ester derived from myristic acid and isopropyl alcohol widely used in cosmetic and pharmaceutical formulations. Unlike the already mentioned sesquiterpenes, this compound is not found in natural vegetal oils (Opdyke, 1976). Cebi et al. (2020) explained that isopropyl myristate compromises essential oils' purity and authenticity, since it highlights adulterations aimed at reducing production costs. According to them, these adulterations can change oils' natural properties. Therefore, the detection of this compound demands assessing the essential oil's authenticity.

Table 3. Chemical constituents of *Chrysopogon zizanioides* essential oil produced in Brazil.

Entry	Compound	Retention Index		Reference
		Experimental[a]	Literature	
1	(3S,3aS,6R,8aS)-3,8,8-Trimethyl-7-methyleneoctahydro-1H-3a,6-methanoazulene; *(Prezizaene)*	1439	1443	Mass Spectra Library: NIST[c]
2	(3S,3aS,6R,8aS)-3,7,7-Trimethyl-8-methyleneoctahydro-1H-3a,6-methanoazulene; *(Khusimene)*	1444	1449	Schwob et al., 2004
3	α-Muurolene	1476	1478	Abella et al., 2000
4	4a,8-Dimethyl-2-(prop-1-en-2-yl)-1,2,3,4,4a,5,6,7-octahydronaphthalene	1482	1492	Mass Spectra Library: NIST
5	(5R,10R)-10-Methyl-6-methylene-2-(propan-2-ylidene) spiro[4.5]dec-7-ene; *(β-Vetispirene)*	1485	1495	Mass Spectra Library: NIST
6	Cyclohexanemethanol, 4-ethenyl-α,α,4-trimethyl-3-(1-methylethenyl)-, [1R-(1α,3α,4β)]; *(Elemol)*	1546	1540	Viswanathan et al., 2002
7	(3aR,6R,8aR)-7,7-Dimethyl-8-methylenehexahydro-1H-3a,6-methanoazulen-3(2H)-one; *(Khusimone)*	1594	1605	Kahriman et al., 2011
8	(1S,2S,4aR,8aS)-2-Isopropyl-4a-methyl-8-methylenedecahydronaphthalen-1-ol; *((+)-Junenol)*	1612	1610	Weyerstahl et al., 1998
9	Selin-6-en-4α-ol	1613	1615	
10	1-Naphthalenol, 1,2,3,4,4a,7,8,8a-octahydro-1,6-dimethyl-4-(1-methylethyl)-, [1R-(1α,4β,4aβ,8aβ)]	1628	1630	Mass Spectra Library: NIST
11	(3S,3aS,6R,7S,8aS)-3,7,8,8-Tetramethyloctahydro-1H-3a,6-methanoazulen-7-ol; *(allo-Khusiol)*	1639	1628	Mass Spectra Library: NIST
12	2-Naphthalenemethanol, decahydro-α,α,4a-trimethyl-8-methylene-, [2R-(2α,4aα,8aβ)]; *(β-Eudesmol)*	1645	1645	Hazzit et al., 2006
13	Cubenol	1649	1647	Ngassoum et al., 2004
14	(2S)-2-((1R,3aR,4R,5S,7aS)-1,7a-Dimethyloctahydro-1H-1,2,4-(epimethanetriyl)inden-5-yl)propan-1-ol	1653	1682	Mass Spectra Library: NIST23s.lib
15	(3S,3aR,6R,8aS)-3,7,7-Trimethyl-8-methylenehexahydro-1H-3a,6-methanoazulen-2(3H)-one; *(Epizizanone)*	1659	1669	Adams et al., 2004
16	Spiro[4.5]dec-8-en-7-ol, 4,8-dimethyl-1-(1-methylethyl); *(Trichoacorenol)*	1663	1665	Mass Spectra Library: NIST
17	(2R,3R,3aR,6R,8aS)-3,7,7-Trimethyl-8-methyleneoctahydro-1H-3a,6-methanoazulen-2-ol; *(Zizanol)*	1666	1665	Mass Spectra Library: NIST
18	(3R,3aR,5S,6R,7aR)-3,6,7,7-Tetramethyloctahydro-3a,6-ethanoinden-5-ol; *(Allocedrol)*	1671	1664	Mass Spectra Library: NIST
19	6-Methyl-2-(4-methylcyclohex-3-en-1-yl)hepta-1,5-dien-4-ol; *(β-Atlantol)*	1687	1665	Mass Spectra Library: NIST
20	1-Naphthalenol, decahydro-1,4a-dimethyl-7-(1-methylethylidene)-, [1R-(1α,4aβ,8aα)]; (Juniper camphor)	1690	1688	Yuping et al., 2008
21	(R)-2-((4aS,8aR)-4a-Methyl-8-methylene-1,4,4a,5,6,7,8,8a-octahydronaphthalen-2-yl)propan-1-ol; (Vetiselinenol)	1717	1723	Lima et al., 2011
22	((3S,3aR,6R,8aS)-7,7-Dimethyl-8-methyleneoctahydro-1H-3a,6-methanoazulen-3-yl)metanol; *(kushimol)*[b]	1736	1735	Massardo et al., 2006
23	(E)-2-((8R,8aS)-8,8a-Dimethyl-3,4,6,7,8,8a-hexahydronaphthalen-2(1H)-ylidene)propan-1-ol; *((E)-Isovalencenol)*[b]	1783	1783	Massardo et al., 2006
24	Nootkatone	1799	1799	Suárez et al., 2005
25	(E)-2-((8R,8aS)-8,8a-Dimethyl-3,4,6,7,8,8a-hexahydronaphthalen-2(1H)-ylidene)propanal	1806	1787	Mass Spectra Library: NIST
26	β-Vetivone	1812	1806	Lima et al., 2011
27	H-Cyclopropa[a]naphthalen-2-one, 1,1a,4,5,6,7,7a,7b-octahydro-1,1,7,7a-tetramethyl-, (1aα,7α,7aα,7bα)	1818	1787	Pripdeevech & Saansoomchai 2013
28	2(3H)-Naphthalenone, 4,4a,5,6,7,8-hexahydro-4,4a-dimethyl-6-(1-methylethylidene)-, (4R-cis) *(α-Vetivone)*[b]	1832	1836	Kobaisy et al., 2002

[a]Calculated Linear Relationship Index; [b]Major compounds; [c] STEIN (2008) and NIST (2008) National Institute of Standards and Technology. Mass Spectral Library (NIST/EPA/NIH).

Table 4. Chemical constituents of *Chrysopogon zizanioides* essential oil produced in China.

Entry	Compound	Retention Index		Reference
		Experimental[a]	Literature	
1	6-Methyltridecane	1311	1316	Cardeal et al., 2006
2	2,5-Dimethyldodecane	1313	1312	Mass Spectra Library: NIST[b]
3	2,3-Dimethylundecane	1315	1312	Mass Spectra Library: NIST
4	2,6,11-Trimethyldodecane	1333	1358	Mass Spectra Library: NIST
5	3,5-Dimethyldodecane	1339	1321	Mass Spectra Library: NIST
6	5-Methyltridecane	1353	1350	Mass Spectra Library: NIST
7	4-Methyltridecane	1358	1356	Mass Spectra Library: NIST
8	2-Methyltridecane	1363	1363	Mass Spectra Library: NIST
9	3-Methyltridecane	1370	1373	Mass Spectra Library: NIST
10	Farnesane	1372	1369	Mass Spectra Library: NIST
11	1H-3a,7-Methanoazulene, 2,3,4,7,8,8a-hexahydro-3,6,8,8-tetramethyl	1406	1421	Mass Spectra Library: NIST
12	(3R,3aR,7R,8aS)-3,8,8-Trimethyl-6-methyleneoctahydro-1H-3a,7-methanoazulene (β-Funebrene)	1413	1414	Adams et al., 2004
13	n-Pentadecane	1421	1497	Mass Spectra Library: NIST
14	cis-Thujopsene[a]	1426	1429	Adams et al., 2005
15	Azulene	1435	1454	Mass Spectra Library: NIST
16	5-Methyltetradecane	1449	1449	Mass Spectra Library: NIST
17	2,6,10-Trimethyltetradecane	1452	1542	Mass Spectra Library: NIST
18	4-Methyltetradecane	1457	1457	Mass Spectra Library: NIST
19	2-Methyltetradecane	1463	1464	Mass Spectra Library: NIST
20	3-Methyltetradecane	1470	1475	Mass Spectra Library: NIST
21	Benzene, 1-methyl-4-(1,2,2-trimethylcyclopentyl)	1500	1505	Mass Spectra Library: NIST
22	1,2,3,5,6,7,8,8a-octahydro-1,4-dimethyl-7-(1-methylethenyl)-, [1S-(1.alpha.,7.alpha.,8a.beta.)] azulene	1502	1498	Mass Spectra Library: NIST
23	Cedrol[a]	1595	1595	Duquesnoy et al., 2006
24	Patchouli alcohol	1650	1647	Mass Spectra Library: NIST
25	Isopropyl myristate[a]	1833	1832	Mass Spectra Library: NIST

[a]Major compounds. [b c] STEIN (2008) and NIST (2008) National Institute of Standards and Technology. Mass Spectral Library (NIST/EPA/NIH).

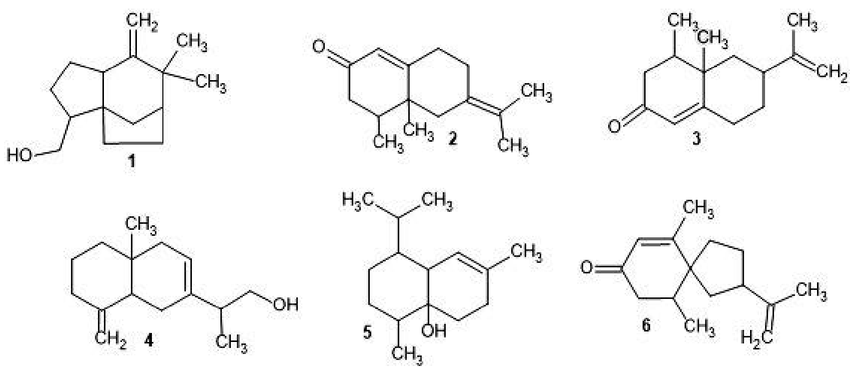


Figure 2: Major chemical components found in the sample of *Chrysopogon zizanioides* essential oil produced in Brazil: khusimol **(1), α**-vetivone **(2),** nootkatone **(3),** vetiselinenol **(4),** cubenol **(5)** and **β**-vetivone **(6)**.

Furthermore, the Chinese oil presented sesquiterpenes characteristic of *C. zizanioides* root extracts such as cis-thujopsene (Gautam e Agrawa 2021), cedrol (Filippi et al., 2013) and pachoulol (Oliveira et al., 2022). However, this species extracts' typical markers are khusimol, β-vetivone, vetiselinenol and β-vetisperene, and they were not detected. The Brazilian oil showed khusimol alcohol concentration slightly above the average often recoded for this compound (3.4% - 13.7%). On the other hand, sesquiterpene β-vetisperene (0.4%) rate was below the average (1.6% - 4.5%). Alcohol vetiselinenol (6.4%) and the ketone β-vetivone (5.2%) rates were within the average range reported in the literature (1.3% – 7.8% and 2.5% – 6.3%, respectively) (Champagnat et al., 2006).

Results pointed towards different chemical composition between the two oils, and it highlights the greater efficiency of the Brazilian oil in comparison to the Chinese one when it comes to giving *Pinus* wood fungal resistance. This finding can be closely related to the presence of three major compounds, namely: khusimol (18.6%), α-vetivone (10.7%) and E-isovalencenol alcohol (2.7%), which were not found in the Chinese oil. Powers et al. (2018) and Soidrou et al. (2013) corroborated this information.

According to Powers et al. (2018), *C. zizanioides* essential oil presented antifungal activity, mainly against *Aspergillus niger*, *Candida albicans* and *Cryptococcus neoformans*. They indicated that such an activity can be partially attributed to the presence of the following major extract constituents: (E)-isovalencenol (13.5%), khusimol (12.1%) and α-vetivone (5.4%). Soidrou et al. (2013) argued that *C. zizanioides* essential oil rich in khusimol (25.6%), α-vetivone (7.7%), bicyclo-vetivenol (11.4%), epi-α-cadinol (5.9%) and nootkatone (5.2%) inhibited the xylophagous fungi *Gloeophyllum trabeum*, *R. placenta*, *Coniophora puteana* and *Coriolus versicolor*. It is worth mentioning that the study by Powers et al. (2018) and that by Soidrou et al. (2013) showed the importance of khusimol and α-vetivone for fungal activity performance.

Despite evidence in the literature and in results of studies on the action of these specific compounds in fungal inhibition, the hypothesis that other compounds may have synergistically contributed to this inhibition cannot be ruled out. This assumption becomes feasible if one bears in mind that *C. zizanioides* essential oil has a complex chemical composition (Davi, et al., 2023). Broda (2020) makes it clear that essential oils' antifungal efficacy often results from the combination of several compounds that can synergistically act to enhance the effect of each other. Furthermore, if one takes into account that khusimol and α-vetivone were not detected in the composition of the Chinese oil, and that antifungal activity was also noticed, although it was less significant, it is possible assuming that other compounds may have given this property to this oil.

Accordingly, compounds cis-thujopsene and cedrol found in the Chinese oil at concentrations of 9.8% and 4.5%, respectively, reinforced the aforementioned hypothesis. Therefore, they are major constituents of this oil and they may have played this role. Thus, Liu et al. (2019) observed that the *Platycladus orientalis* heartwood extract showed significant inhibitory effects on xylophagous fungi, which is accountable for white rot (*Ipex lactenus*) and brown rot (*Gloeophyllus trabeum*). This finding can be justified by the fact that this extract is mainly composed of cis-thujopsene and cedrol.

However, although it is not possible pinpointing the exact mechanism involved in *R. placenta* development's inhibition by *C. zizanioides* essential oil, it is known that essential oils hold compounds that interact with cell membrane, change its permeability and lead to cell lysis (Wozniak, 2022). They reported that these compounds can interfere with the cell wall essential components synthesis and, consequently, impair cell wall ability to absorb nutrients.

It is worth noticing that, despite *C. zizanioides* essential oil property in giving fungal resistance to *Pinus* wood, Broda (2020) explains that, for a natural product to be effectively used for this purpose, advanced research must be carried out to clarify several aspects, among them one finds compounds' leaching and biodegradation under real wood use conditions; selective toxicity to different fungi species; and natural compounds/polymers combinations, in order to enhance the effect of the natural product.

According to Chaudhari et al. (2021), the main actions to improve essential oils' efficacy, mainly its antifungal activity, include nanoencapsulation and nanoemulsion formulations. The aim of these approaches is to improve essential oils' stability, water solubility, controlled release and persistence, as well as to overcome their limitations such as susceptibility to oxidation and low solubility, as mentioned by David et al. (2023), since those are *C. zizanioides* essential oil features. Broda (2020) and Chaudhari et al. (2021) considerations point out important research lines aimed at *C. zinazoides* oil and at optimizing its ability to protect wood against fungal deterioration processes.

CONCLUSIONS

Fresh pine wood is highly susceptible to wood-eating fungus *R. placenta*. However, it became resistant to this fungus under laboratory conditions, when it was treated with the Brazilian *C. zizanioides* essential oil at the following concentrations: 25%, 50% and 100%. On the other hand, it only became partially resistant when it was treated with the Chinese oil. The Brazilian oil has compounds typical of *C. zizanioides,* namely: khusimol, β-vetivone, vetiselinenol and β-vetisperene. The Chinese oil, in its turn, only holds few sesquiterpenes, among them, cis-thujopsene, cedrol and pachoulol. Its major component is isopropyl myristate, which does not have plant origin.

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AUTHORSHIP CONTRIBUTION

Project Idea: HT

Funding: HT

Database: VJF; HT

Processing: VJF; CERS; HT

Analysis: VJF; CERS; HT

Writing: HT;

Review: VJF; CER

DATA AVAILABILITY

The datasets analyzed during the current study are available from the corresponding author upon reasonable request.

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