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Essential oil of *Piper macedoi* Yunck. leaves, potential alternative for the management of banana anthracnose disease

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ABSTRACT

The fungus *Colletotrichum musae* could cause a disease called anthracnose which reduces the quality of the banana fruit in the postharvest period. Anthracnose causes rots, peel lesions and generate great economic losses. Synthetic fungicides, used as an attempt to reduce the pathogen population, although effective, when used in amounts and frequency above the recommended, cause damage the environment and human health and can induce resistance. Research has focused in the search for alternative methods to reduce the use of synthetic substances in managing of agricultural diseases. The control of pathogens using compounds of natural origin is highlighted among new management techniques. Thus, we aimed to extract and characterize the essential oil from *Piper macedoi* leaves and evaluate its effect in the inhibiting the mycelial growth of the fungus *C. musae* in vitro. The essential oil was extracted by hydrodistillation, analyzed and characterized by Gas Chromatography and submitted to assays of fungicidal activity with *C. musae*. In comparison with dry plant material, yield of *P. macedoi* essential oil was 0.93 %. Piperitone (26.48 %), silvestrene (13.03 %), and bicyclogermacrene (10.45 %), were the major compounds identified in the essential oil composition considering the percentage in area. Regarding the antifungal activity tests, the essential oil showed a low inhibitory concentration (IC) for the IC50 (0.804 $mL.L^{-1}$) and IC90 (1.80 $mL.L^{-1}$), showing that the essential oil from *P. macedoi* leaves has great potential to control *C. musae*.

1. Introduction

Colletotrichum musae (Berk. & M.A. Curtis) Arx is an important cosmopolitan pathogenic fungus, which causes anthracnose on banana fruit. The postharvest stage of the crop is the most affected by the pathogen, with damages from the visual aspect of the ripe fruit, to the final processes of the banana's productive chain, the commercialization (Singh and Tripathi, 2015). An aggravating is that although fruit may not present symptoms at the moment of the harvest, they can manifest in the postharvest, causing substantial percentage of losses (Helin et al. 2017).

The type of market to which the banana plantation belongs determines its postharvest treatment. Local markets do not struggle with the postharvest stage, because they quickly sell the fruit (within 5 days), while distant markets require special attention. The banana harvest point must happen before complete ripening, as it happens quickly and irreversibly (Lundgren et al. 2022). After the beginning of ripening, the fruit undergoes significant changes in physical and chemical attributes (such as conversion of starch into sugar, softening of the pulp, aroma, flavor, and pigmentation of the peel), which provide a relatively short shelf life to the fruit (about 6–8 days under environment conditions) (Aquino et al. 2016).

To extend the quality of the bananas, right after the harvest, the fruit need to be washed with water, and then sprayed or dipped into solutions of synthetic fungicides before packing (Vilaplana et al. 2018). The main negative impacts of the use of synthetic fungicides are: damage to the environment, risk to human and animal health due to the maintenance of residues in plants, and resistance to pathogens (Ngibad et al. 2021; Chanthini et al. 2018). With the increase in strictness

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regarding maximum levels of pesticide residues in banana fruits in the main importing markets, pressure has been increasing on producers and exporters, since products that do not meet these new requirements cannot be exported (FAO, 2022). Essential oils have been recognized as important natural sources of compounds with phytosanitary activity with a wide range of applications (Hammoud et al. 2022). Studies report the potential of essential oils to control phytopathogens through fungistatic action, mycelial inhibition, and spore germination (Kumar and Kudachikar, 2018; Madjouko et al. 2019).

Botanical insecticide are gaining substantial commercial interest as an important alternative to synthetic chemical pesticides in pest and disease management programs. (Senthil-Nathan, 2015; Kumar et al. 2019). Another interesting alternative is biological control, such as entomopathogenic fungi, capable of colonizing different types of pests. These fungi emerge as a viable option in the management of agricultural pests (Karthi et al. 2019; Kalaivani et al. 2021). Essential oils, which are compounds generated in the secondary metabolism of plants, also have been attracting the attention of the phytosanitary chemical industry. In particular, the chemical compounds present in plants of the Piperaceae family have been highlighted because they are biodegradable products and have multiple biological effects such as insecticides, fungicides, molluscicides, acaricides and bactericides (Piton et al. 2014; Rapado et al. 2014; Božović et al. 2015; Oliveira et al. 2016; Benchimol et al. 2017; Madhumita et al. 2019; Herath et al. 2019).

The Piperacae family comprises species with considerable chemotypic diversity (Madhumita et al. 2019). The importance of this family is due to its biological and medicinal applications (Arunachalam et al. 2020). *Piper aduncum* stands out among the species from the family due to its potential to control several insects from different orders as Diptera, Hymenoptera, Coleoptera, Hemiptera among others (Piton et al. 2014). Nevertheless, other species from the family also show potential to promote biological activities.

The species *Piper macedoi* Yunck. is native and endemic in Brazil and has a lot of similarity with the species *Piper aduncum*, which is well known and studied (Christ et al. 2016). There are few works on the chemical composition of the essential oil from the leaves of *P. macedoi* (Oliveira et al. 2016) and reports on the application of this oil to control pests and diseases are rare. So far, its use in the control of fungi has not been disclosed. In this scenario, we carried out the determination of the chemical composition of the essential oils extracted from the leaves of *P. macedoi* and the evaluation of the fungicidal and fungistatic potential of the essential oil on the mycelial growth of the fungus *C. musae*, responsible for the anthracnose disease in bananas.

2. Material and methods

2.1. Plant material

Branches with leaves from *P. macedoi* were harvested in the morning (in April 2019), at the edge of a forest fragment in regeneration located in the district of Rive, municipality of Alegre-ES/Brazil at the following geographic coordinates: latitude 20°45'19″S and longitude 41°27'04″W. The material was dried in an air forced circulation oven (Marconi mod. MA035/5) at a temperature of 40 °C until the mass remained constant, to standardize the dehydration of the leaves. After drying, the leaves were weighed, packed into plastic bags, and stored in a freezer at −10 °C, until the extraction of the essential oil.

Piper macedoi exsiccates were pressed according to the usual herborization techniques and the species were deposited at the Capixaba Herbarium (CAP), located at the Federal University of Espírito Santo, in Alegre - Brazil (register number: 42140).

2.2. Essential oil extraction

The essential oil extraction was carried out with the hydrodistillation technique using a Clevenger type apparatus according to Santos et al. (2021). The dry plant material and distilled water were added into a 2 L round bottom flask. The distillation occurred for 4 h After the extraction, the hydrolate (water and essential oil) was collected and subjected to centrifugation at 6000 RPM, for 10 min. The essential oil was separated from the aqueous medium and weighed for the extraction yield determination (% $w.w^{-1}$, based on dry biomass).

The determination of the refractive index and the measurements of average density (g/cm^3) of the essential oil were performed in an acclimatized room at 20 ºC. A volume of 1.00 mL of essential oil was weighed on a precision analytical balance (± 1 mg), to determine the density (Shimadzu AUY 220). The determination of the refractive index was performed by a bench-top Abbé refractometer (Quimis Q767B). These procedures were repeated 5 times for both determinations.

2.3. Chemical characterization of the essential oil

The essential oil was analyzed by gas chromatography with flame ionization detector (GC-FID) (Shimadzu GC-2010 Plus) and by gas chromatography coupled to mass spectrometry (GC-MS) (Shimadzu QPMS-2010) according to Santos et al. (2021).

The following chromatographic conditions were used for both analyzes: fused silica capillary column (30.00 m x 0.25 mm) with Rtx®−5MS stationary phase (0.25 μm film thickness); N_2 (in GC-FID analysis) and He (in GC-MS analysis) as carrier gases with a flow rate of 3.0 $mL.min^{-1}$; initial oven temperature of 40 °C with gradual increase of 3 °$C.min^{-1}$ until it reached 240 °C; injector temperature of 250 °C; detector temperature of 280 °C; split ratio of 1:30. The analyses of gas chromatography coupled to mass spectrometry were performed in an equipment operating by electronic impact with 70 eV impact energy; scan speed of 1000; scanning interval of 0.50 $fragments.sec^{-1}$ and detected fragments from 29 to 400 ($m.z^{-1}$).

The compounds were identified through the comparison between their mass spectra with those available in the database of the Willey7, NIST05, and NIST05s spectrotecs, with the co-injection of standards and with the calculated Retention Index (RI). For the RI calculation, a mixture of linear n-alkanes (C7 to C40) was used. The calculated RI was compared to data described in the literature (Adams, 2007; NIST, 2011).

The relative percentages of each compound were calculated based on the relative area of the corresponding chromatographic peaks by the analysis performed through GC-FID, considering the compounds with relative area greater than 1 %.

2.4. Antifungal assay of essential oil on the growth of Colletotrichum musae

The isolate of *Colletotrichum musae* (Berk. & M.A. Curtis) Arx (strain code: CCF 243) used in the study was friendly obtained by the Department of Phytopathology of the Federal University of Viçosa (UFV) - Campus of Viçosa (Brazil). To verify the effect of the essential oil, in vitro tests were carried out with several dilutions on the mycelial growth of the pathogen.

For the growth test, aliquots of pure essential oil were added to the BDAculture medium, and poured into a 90 mm diameter Petri dish at the final concentrations of 1.0 $mL.L^{-1}$, 1.5 $mL.L^{-1}$, 2.0 $mL.L^{-1}$ and 2.5 $mL.L^{-1}$ of the essential oil. The fungicide thiabendazole, in its commercial presentation Tecto SC® (Syngenta), was used as positive control, under the dosage recommended by the manufacturer (0.65 $mL.L^{-1}$), because it is the standard product used in banana postharvest. For the negative control, pure BAD culture medium was used.

After solidification of the culture medium with the study concentrations, a 0.5 cm disk of the fungal colony previously cultivated for seven days (at a concentration of 7×10^4 conidia/mL) was added to the center of the Petri dish, which was subsequently sealed and incubated in a growth oven of the type BOD (Biochemical Oxygen Demand) at 25 °C, under a 12-hour light/dark photoperiod for 7 (seven) days. After that period, two opposed measurements of mycelial growth were taken,

with the aid of a caliper graduated in centimeters, to analyze the percentage of inhibition. For the calculation of the percentage of mycelial growth inhibition (PMGI), we applied the methodology used by Pereira et al. (2013) with small adaptations.

2.5. Statistics

The completely randomized design was used with four concentrations of essential oil, a positive and a negative control, in triplicate. The data were subjected to analysis of variance by the F test at 1 % significance. Averages were compared by the Tukey test at 5 % significance. Then, they were submitted to a Probit regression using the R software (R Development Core Team, 2017) to determine the IC50 and IC90 (with a 95 % confidence interval), for the essential oils from *P. macedoi* leaves in the inhibition of the mycelial growth of *C. musae*, according to Carvalho et al. (2017).

3. Results

3.1. Piper macedoi essential oil

The hydrodistillation process provided a slightly viscous essential oil, with light pale green color, density of 0.913 g.cm^{-3}, refractive index of 1.477, pleasant odor, and an average extraction yield of 0.93 % (w/w) in comparison to the dry mass.

GC-FID and GC-MS identify 16 compounds with area greater than 1 % with piperitone (26.48 %), silvestrene (13.03 %), and bicyclogermacrene (10.45 %) as majors components (Fig. 1; Table 1).

In this essential oil, it was observed that the class of monotepenes (monoterpenes hydrocarbons = 32.53 % and monoterpenes oxygenated = 34.64 %) was more expressive than sesquiterpenes class (sesquiterpenes hydrocarbons = 27.55 % and sesquiterpenes oxygenated = 5.27 %) as shown in Table 1.

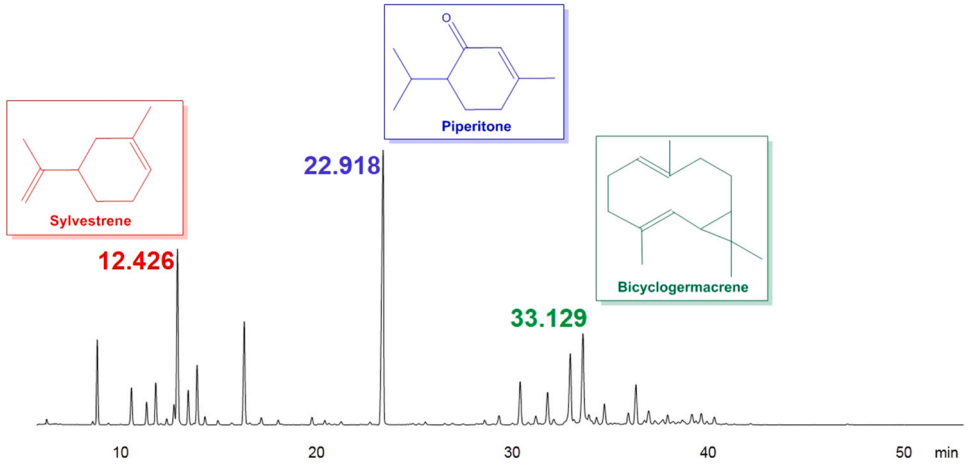

Fig. 1. Chromatogram (CG-FID) with retention time and chemical structure of major compounds (compounds with relative area greater than 10 %).

Table 1
Identification of the compounds from the essential oils through Retention Index and Mass Spectrometry (GC/MS)[a].

Compound	CAS numbers[b]	Retention time (min)	Calculated retention index[c]	Theoretical retention index[d]	Relative areas (%)[e]
alpha-pinene	80–56–8	8.332	931	932	5.34
beta-pinene	127–91–3	10.076	973	974	2.70
myrcene	123–35–3	10.852	992	988	1.60
alpha-phellandrene	99–83–2	11.319	1003	1002	3.02
sylvestrene	1461–27–4	12.426	1027	1025	13.03
(*Z*)-β-ocimene	3338–55–4	12.977	1036	1032	2.54
(*E*)-β-ocimene	3779–61–1	13.431	1047	1044	4.31
linalool	78–70–6	15.835	1102	1095	8.16
piperitone	89–81–6	22.918	1252	1249	26.48
trans-caryophyllene	87–44–5	29.920	1417	1417	4.04
alpha-humulene	6753–98–6	31.321	1451	1452	3.26
germacrene D	23986–74–5	32.481	1479	1484	7.72
bicyclogermacrene	67650–90–2	33.129	1495	1500	10.45
delta-cadinene	483–76–1	34.227	1523	1522	2.08
(*E*)-nerolidol	40716–66–3	35.840	1565	1561	3.71
caryophyllene oxide	1139–30–6	36.487	1582	1582	1.56
Monoterpenes hydrocarbons					32.53
Monoterpenes oxygenated					34.64
Sesquiterpenes hydrocarbons					27.55
Sesquiterpenes oxygenated					5.27
Total compounds in the essential oil with a relative area greater than 1 %					100.00

[a] The compounds were indentified by the Retention Index (GC/FID) and Mass Spectrometry (GC/MS) using a Rtx ®−5MS column. [b]CAS (Chemical Abstract Service) numbers queried at NIST. [c]Retention index calculated from data obtained by sampling of saturated n-alkanes (C7-C40). [d]Theoretical retention index obtained in the literature of Adams (2007), NIST (2011), [e]Compounds with relative areas > 1 % were identified.

Table 2
Percentage of inhibition of the mycelial growth (± EP) of *C. musae* at different concentrations of the essential oil from *P. macedoi* leaves, under laboratory conditions.

Concentrations (mL.L^{-1})	Mycelial Growth Inhibition (%)
0.65[1]	100.00 a[2]
2.50	96.67 ± 0.833 a
2.00	91.67 ± 0.833 ab
1.50	84.17 ± 2.204 b
1.00	63.33 ± 3.333c
0.00	0 d
CV (%)	4.81 %

[1] Thiabendazole 485 g/L. [2] Means followed by the same letters are similar, by the Tukey's test at 5 % probability.

3.2. Test of essential oils on the mycelial growth of C. musae

The results of Table 2 show that essential oil concentrations above 2.0 mL.L^{-1} promoted an inhibition of the mycelial growth of the fungus *C. musae* greater than 90 %. The essential oil used at the dosage of 2.0 mL.L^{-1} did not differ statistically from the positive control, in which the commercial fungicide thiabendazole was used. Therefore, the results confirm that the compounds present in this essential oil are effective in inhibiting the fungus *C. musae*. We observed that the inhibition of *C. musae* mycelial growth, after 7 days of inoculation in BDA medium, showed significant differences between treatments, in each of the concentrations (Table 2).

The normality of the residues, through the Shapiro-Wilk test, at 5 % of significance, proved homogeneity of variances, with p (normal) greater than 5 %. This allowed us to carry out an analysis of variance that confirmed the existence of significant difference between treatments, presenting F = 452.56 and p > 0.001 in the test with *P. macedoi* essential oil.

The Tukey's test showed significant differences between treatments, indicating that the essential oil is more efficient at the concentration of 2.5 mL.L^{-1} (Fig. 2). Considering the criterion of inhibition above 80 %, it appears that the concentrations of 1.5 mL.L^{-1} are satisfying. The oils were submitted to a second stage of tests, and their lethal concentrations were estimated through the Probit regression analysis.

Through the Probit regression, regarding the percentage of mycelial growth inhibition, we found that the response dose for the essential oil of *P. macedoi* leaves is relatively high in low concentrations, demonstrating its potential to be used in the management of *C. musae* (Table 3).

The inhibitory concentration of the essential oil of *P. macedoi* on the mycelial growth of *C. musae* is shown in Fig. 3. Within a 95 % confidence interval, the IC50 was of 0.804 mL.L^{-1} and the IC90 of 1.80 mL.L^{-1}, so we verified the occurrence of data adjustments to the analysis with Pearson's Chi-square equal to 7.19 and p-value equal to 0.1082 a standard normal distribution ϕ.

4. Discussion

The control of anthracnose in the postharvest of bananas plantations has been an issue for producers, due to the reduction in fruit quality caused by the disease, affecting consumer acceptance and market value. The results of a plantation affected by anthracnose will not only make commercialization difficult, but it will also significantly reduce the production yield (Singh and Tripathi, 2015).

The concern with the environment and human health is noticeable, triggering the search for alternative methods other than the use of agrochemicals to control plant diseases. Considering the multiple biological effects presented by species from the Piperaceae family, this study investigated the efficacy of the essential oil from *P. macedoi* leaves, a native and endemic species in Brazil, because there is lack of studies with this species.

Oliveira et al. (2016) identified chemical compounds in the essential oil of *P. macedoi* leaves harvested in Minas Gerais, Brazil. In their work compounds were distributed among: monoterpenes as trans-β-ocimene and β-phelandrene (28.5 % and 9 % of relative area respectively); oxygenated sesquiterpenes as germacrene-D, cubedol and nerolidol (3.7 %, 1.5 % and 1.4 %, respectively) and arylpropanoids, highlighting sarisan, myristicin, safrole and dilapiol (14.1 %, 5.0 %, 4.1 %, and 1.1 %, respectively) (Oliveira et al. 2016). This is one of the first results concerning the extraction and characterization of *P. macedoi* essential oil reported in the scientific literature.

In the present study the major compounds identified in *P. macedoi* essential oil were two arylpropanoids (piperitone with 26.48 % and silvestrene with 13.03 %), and one hydrogenated sesquiterpene (bicyclogermacrene with 10.45 %). Comparing the results presented in our study with the only characterization study of this plant published so far (Oliveira et al. 2016), we observed that the authors did not identify any arylpropanoid or hydrogenated sesquiterpene compounds among the major ones.

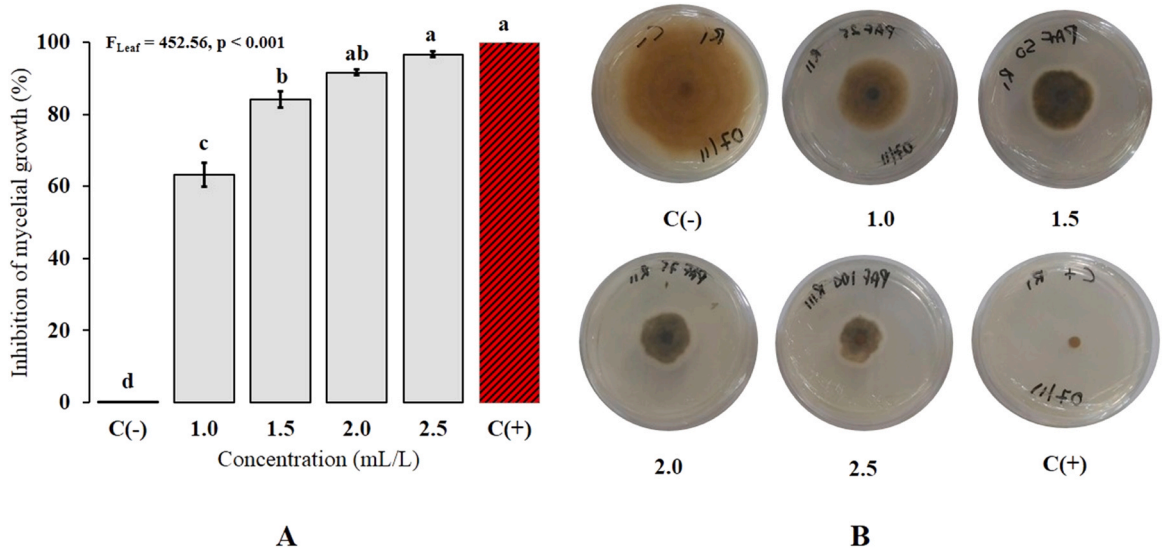

Fig. 2. A: Inhibition of the average mycelial growth of *C. musae*, under different concentrations of the essential oil from *P. macedoi* leaves. Bars accompanied by the same letter do not differ by the Tukey's test at 5 % probability. **B:** Images of the fungicidal activity test with the variation of the essential oil concentration.

Table 3
Response concentration of the Essential oil (EO) from *P. macedoi* leaves and roots in the inhibition of the mycelial growth of *C. musae*.

Treat.	Inclination (± EP)	[a,b]IC50 mL.L^{-1}	[a,b]IC90 mL.L^{-1}	X^2	DF	p-value
EO_F	3.631 ± 0.24	0.804 (0.69–0.90)	1.80 (1.69–1.98)	7.19	13	0.1082

[a] IC: inhibitory concentration; [b] 95 % confidence interval; X^2: Chi-square; DF: Degree of Freedom

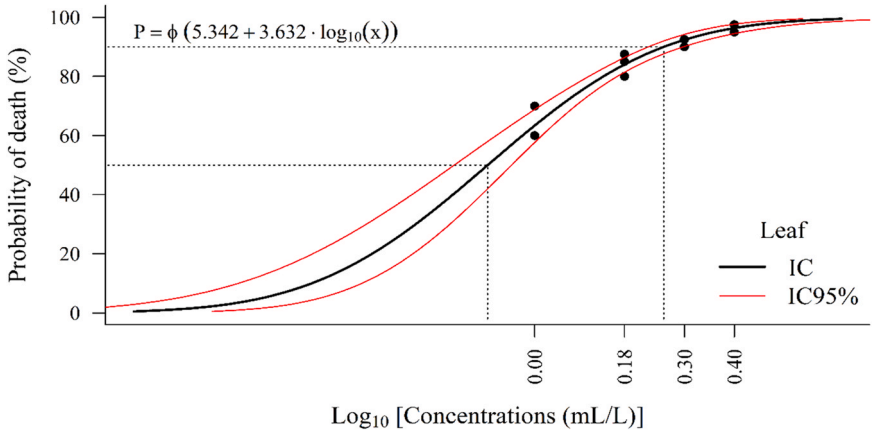

Fig. 3. Inhibitory concentration of the essential oil from *P. macedoi* leaves on *C. musae*. Concentrations transformed by $\log_{10}(x)$.

In general, the relative areas of the major compounds were very different in these studies, suggesting chemotypic diversity between the species and possible effects of the environment, considering that the plants are located in different regions, which implies differences in soil, climate, and altitude.

Although botanically identical, chemical differences in the essential oils of plants, even in the same species, often occur (Bruni et al., 2015; Khammassi et al. 2018; Li et al. 2020). Intrinsic factors (such as the occurrence of specific chemotypes and the stage of development of the plant) affect these differences, both qualitatively and quantitatively. Some extrinsic factors may also interfere with the chemical variability of the vegetable as the temperature, the relative humidity of the air, the luminosity and the humidity and fertility of the soil. Seasonal and spatial effects must be considered, since they have a great influence on the chemical composition and quantity of compounds in the essential oils (Gobbo-Neto et al. 2017). The pattern of chemical variability is unpredictable, as each species reacts differently to the variations.

Authors have already tested some of the compounds present in the composition of the essential oil from *P. macedoi* leaves, as nerolidol, piperitone an linalool, in isolation, and could identify fungicidal evidence (Abdelgaleil et al. 2008; Yaguchi et al. 2009; Božović et al. 2015; Chan et al. 2016). Linalool even presented synergistic effects when associated with other essential oils (Herman et al. 2016).

Products from the agri-food industry are frequently contaminated by fungal and bacterial infestations. In order to ensure food safety, there is a need for control measures that are effective against these contaminations. The volatile components present in essential oils have been frequently reported as possessing strong fungicidal and antimicrobial potential (Elshafie, 2022; Kapustová et al. 2021; Ibáñez and Blázquez 2021). The main obstacles to the application of essential oils are due to their volatility, low water solubility, low bioavailability, low long-term stability and marked organoleptic effects (Barradas and de Holanda e Silva, 2021; Falleh et al. 2020). An alternative would be the preparation of emulsion or encapsulation of the essential oil that can provide a longer and more effective use of the biological effect with the controlled release that also restricts its strong characteristic odor (Torshabi et al. 2023; Kaboudi et al. 2023).

Studies report the fungicidal, antioxidant, and microbial effects of essential oils containing piperitone in its majority composition (relative area of 27.6 %) (Božović at al. 2015; Valadares et al. 2018). Chemical compounds of an essential oil can penetrate the fungal cell membrane and interfere with the metabolic pathway of cell wall synthesis, reducing fungal germination and growth (Nazzaro et al. 2017). The antifungal activity of piperitone isolated from the essential oil of *Matricaria recutita* and *Eucalyptus dives*, inhibited the synthesis of 3-acetyl-deoxynivalenol, and decreased the mRNA levels of the coding proteins Tri4, Tri5, Tri6, and Tri10, necessary to the biosynthesis of deoxynivalenol, a mycotoxin that belongs to the trichothecenes family (Yaguchi et al. 2009). Mycotoxins are produced by different types of molds and fungi and are considered a problem of contaminants in food (Katsurayama and Taniwaki, 2017).

The essential oil from *Artemisia judaica* L. also presents piperitone, as it major compound, and it exhibited moderate to high activity against the pathogenic fungi *Pythium devaryanum*, *Fusarium oxysporum*, *Rhizoctonia solani,* and *Botrytis fabae* (Abdelgaleil et al. 2008).

In vitro tests using thyme essential oil to control the mycelial growth of *Colletotrichum musae,* in organic banana, showed that treatment with a concentration of 500 µL.L^{-1} inhibited the severity of diseases in fruits treated with the essential oil (Vilaplana et al. 2018). In a study by Idris et al. (2015), the essential oils of basil (0.10 %, 0.15 % and 0.20 % v/v), cinnamon (0.025 %, 0.05 % and 0.075 % v/v) were used) and rosemary (0.20 %, 0.25 % and 0.30 % v/v) in vitro tests to control the fungus *C. musae*, a significant inhibition of mycelial growth was observed in all treatments after 7 days of incubation at 25 °C. Clove essential oil at a concentration of 0.1 µL.mL^{-1} completely disrupted conidial germination and mycelial growth of *C. musae* in in vitro experiments (Rizwana, 2018). An in vitro study using various oils was carried out for the management of banana postharvest rot. At a concentration of 1000 ppm, cinnamon oil (*Cinnamomum verum*) showed the lowest radial growth and the highest percentage of growth inhibition (1.67 mm and 98.15 %), followed by mustard oil (*Brassica oleracea*) (54.00 mm and 40.00 %), neem oil (*Azadirachta indica*) (55.17 mm and 38.70 %), castor oil (*Ricinus communis*) (55.83 mm and 37.96 %) and coconut oil (*Cocos nucifera*) (61.17 mm and 32.04 %). (Gairhe et al. 2021). The essential oil of *Conyza bonariensis* inhibited the mycelial growth of C. musae isolates in a laboratory environment and the exposure of conidia to the oil at a concentration of 0.6 µL.mL^{-1} resulted in high percentages of conidia with damaged cytoplasmic membrane and without activity enzymatic (Lundgren et al. 2022).

The fungicidal properties of the essential oil from *Zanthoxylum fagara*, with germacrene D as the major compound (21.1 % of relative area), showed an inhibiting effect against the pathogen *Colletotrichum acutatum* (EC50 = 0.154 $mL.L^{-1}$) (Prieto et al. 2011). The essential oil from *P. macedoi* leaves in the present study also presented this substance in its composition (7.72 %).

A more detailed biochemical study is necessary to elucidate the mechanisms that are prompted when the compounds present in the essential oil and cell target molecules in the fungus interact. What is already knows is that when the essential oil gets in contact with the fungus cells, a rupture in the cells membrane and several changes in ion channels happen (Oz et al. 2015).

This interaction among essential oils and fungus increases the permeability of the membrane. As terpenes and phenolic compounds are hydrophobic and accumulate in the lipid environments of the fungus cells, the fluidity and permeability of the fungus membranes enlarge creating structural damages, releasing vital intracellular constituents and inhibiting the action of enzymes (Di Pasqua et al. 2007; Oz et al. 2015; Katsurayama and Taniwaki, 2017). The essential oils tested on the growth of *C. musae* were efficient to control the fungus. As it is a product of plant origin and easily accessible, the result is attractive from the point of view of food security, providing consumers with fruit that are free of synthetic pesticide residues.

The major compounds, their synergistic activity or even compounds in smaller amounts may have contributed to the efficient inhibition of the mycelial growth of *C. musae* in the present study. Additional studies may be carried out in order to delineate the antifungal activity found here. Nevertheless, our findings have proved the potential of the studied species and we believe this study may contribute to the researches seeking for alternative methods to control anthracnose, reducing the use of agrochemicals.

5. Conclusions

The chemical prospecting carried out with the essential oil of *P. macedoi* leaves, showed a higher percentage of compounds in the class of hydrocarbon and oxygenated monoterpenes, which together reached 67.18 % in relative area of the essential oil composition. The major compounds were the oxygenated monoterpene piperitone, the hydrocarbon monoterpene silvestrene and the hydrocarbon sesquiterpene bicyclogermacrene.

The in vitro bioassay results carried out with the phytopathogen *C. musae* and the essential oil of *P. macedoi* leaves showed an efficient inhibition of the mycelial growth of the fungus at the inhibitory concentrations IC50 of 0.804 $mL.L^{-1}$ and IC90 of 1.800 $mL.L^{-1}$. Both inhibitory concentrations are promising, since natural products offer the possibility of controlling the disease in low amounts of the active ingredient.

Data availability

Data will be made available on request.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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