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Drying temperature affects the quantity and quality of the essential oil of *Psidium* species and contributes to phytotoxicity in model plants

Temperatura de secagem afeta a quantidade e a qualidade do óleo essencial de espécies de *Psidium* e contribui com a fitotoxicidade em plantas modelo

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ABSTRACT

The genus *Psidium* is recognized for its economic value and the species that produce essential oils with notable biological activities. This study investigated the characteristics of the essential oil from the leaves of *Psidium myrtoides* and *Psidium cattleyanum* under different drying temperatures. We aimed to understand how drying temperatures affect the yield and composition of the essential oil, as well as its biological activity. The oils obtained from the leaves of *P. myrtoides* and *P. cattleyanum* dried in an oven at 40°C showed the highest yields (0.86% and 1.07%, respectively). β-caryophyllene was the major compound in all essential oils of *P. myrtoides* and *P. cattleyanum*, except in the oil extracted from *P. myrtoides* leaves dried at room temperature, where the major compound was α-bisabolol (14.46%). Different phytotoxic effects were observed using the emulsion of these oils in bioassays with *Lactuca sativa* and *Sorghum bicolor*, which were associated with the chemical composition and synergy of the identified compounds. The essential oil emulsion from leaves dried at room temperature of both species showed greater phytotoxic activity in the bioassays. Thus, optimizing drying conditions to maximize yield and synergy of compounds from the essential oils of *P. myrtoides* and *P. cattleyanum* is an important step in developing environmentally friendly natural agrochemicals.

Index terms: Bioassays; chromatography; volatile compounds.

RESUMO

O gênero *Psidium* é reconhecido pelo seu valor econômico e por suas espécies produtoras de óleos essenciais, com notáveis atividades biológicas. Este estudo investigou as características do óleo essencial das folhas de *Psidium myrtoides* e *Psidium cattleyanum* sob diferentes temperaturas de secagem. O objetivo foi entender como as temperaturas de secagem afetam o rendimento e a composição do óleo essencial, além de sua atividade biológica, utilizando bioensaios com *Lactuca sativa* e *Sorghum bicolor* para avaliar os efeitos fitotóxicos. Os óleos obtidos das folhas de *P. myrtoides* e *P. cattleyanum* secas em estufa a 40°C apresentaram os maiores rendimentos, sendo 0,86% e 1,07%, respectivamente. O β-cariofileno foi o composto majoritário com maior área relativa em todos os óleos essenciais de *P. myrtoides* e *P. cattleyanum*, exceto, no óleo extraído de folhas secas em temperatura ambiente de *P. myrtoides*, cujo composto majoritário foi o alfa-bisabolol (14,46%). Assim como a alteração do perfil químico dos óleos essenciais, houve diferentes efeitos fitotóxicos utilizando a emulsão desses óleos. Esses efeitos, foram associados a composição química e a sinergia destes compostos. A emulsão de óleo essencial de folhas secas à temperatura ambiente de ambas as espécies mostrou maior atividade fitotóxica nos bioensaios. Assim, a otimização das condições de secagem para maximizar o rendimento e a sinergia dos compostos dos óleos essenciais de *P. myrtoides* e *P. cattleyanum* é um passo importante no desenvolvimento de agroquímicos naturais e sustentáveis.

Termos para indexação: Bioensaios; cromatografia; compostos voláteis.

Introduction

The genus *Psidium* belongs to the Myrtaceae family and is distributed throughout the tropics and subtropics of the Americas and Australia, including Brazil (Fernandes et al., 2021). This genus is recognized for its species producing essential oils (EOs), such as *Psidium myrtoides* O. Berg (MYR), endemic and native to the Atlantic Forest (Tuler et al., 2019). In recent years, the number of studies on the EO of MYR has increased due to its promising biological activities. Vasconcelos et al. (2019) identified around 38 compounds in its EO, 77.6% sesquiterpenes and 9.4% monoterpenes. Additionally, the essential oil from MYR leaves exhibited anticancer (Macedo et al., 2021), antimicrobial, and moderate antiproliferative activities against *Streptococcus mitis*, *S. sanguinis*, *S. sobrinus*, and *S. salivarius* (Dias et al., 2019).

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Similarly, *Psidium cattleyanum* Sabine (CAT)from the same family is known for its fruits used in the food industry, whose bioactivities are attributed to phenolic compounds, minerals, fatty acids, sugars, and carotenoids (Pereira et al., 2018). Besides presenting antioxidant, antifungal, and antimicrobial activities (Chrystal et al., 2020; Rocha et al., 2020; Macedo et al., 2021), these compounds also have larvicidal, herbicidal, and allelopathic activities (Mendes et al., 2023; Alves et al., 2023; Vasconcelos et al., 2019). The EO from CAT leaves also exhibits anti-inflammatory and antinociceptive action, standing out for its potential in the pharmaceutical industry (Silva et al., 2023).

EOs are secondary metabolites extracted from various parts of plants, characterized by a complex chemical composition that provides adaptive advantages to plants in their specific environments (Kumar et al., 2023). Factors such as genotype, plant developmental stage, and environmental conditions influence the synthesis and composition of EOs (Lotfi et al., 2024).

The quantitative and qualitative variation of EOs is dependent on different temperatures, drying methods, and extraction processes employed, especially concerning aromatic species with volatile substances (Caputo et al., 2022; Da Silva et al., 2022; Mokhtarikhah, Ebadi, & Ayyari 2020; Soltanbeigi et al., 2020). This drying of aromatic and medicinal plants aims to minimize the loss of active principles, delay deterioration, and preserve plants for subsequent commercialization and use.

The temperature during drying significantly influences the yields and composition of EOs, as high temperatures can lead to the loss of volatile compounds (Caputo et al., 2022). In addition, different drying temperatures alter the chemical profiles of EOs, potentially resulting in the loss or increase of some compounds due to processes such as oxidation, glycoside hydrolysis, and esterification (Beigi, Torki-Harchegani, & Ghasemi Pirbalouti, 2018).

In this context, this study aimed to evaluate the yield and quality of EOs extracted from leaves of *P. myrtoides* and *P. cattleyanum* subjected to different drying temperatures. Furthermore, we investigated for the first time the phytotoxic effects of these different oils in bioassays with the eudicot *Lactuca sativa* L. and the monocot *Sorghum bicolor* L.

Material and Methods

Plant Material

Leaves from *P. myrtoides* (MYR) (20°45'49''S, 41°31'57''W) and *P. cattleyanum* (CAT) (20°45'48''S, 41°32'2''W) were collected in the municipality of Alegre, in the state of Espírito Santo, Brazil, during the summer (February 2023, average temperature and precipitation of 25.8°C and average 0.469 mm, respectively). Leaves were taken from a single tree at breast height (1.6 m) and around the diameter of the canopy, between 7 a.m. and 8 a.m. on three different days. The voucher specimens (A. C. Tuler 510 and A. C. Tuler 9171 for MYR and CAT, respectively) have been deposited at the Herbarium CAP - Federal University of Espírito Santo. The number of National Management System Genetic Heritage and Associated Traditional Knowledge is AD139DE. The extraction of the oils was conducted in the Vegetable Sample Preparation laboratory, and the plant bioassays were performed in the Plant Cytogenetics laboratory of the Center for Agricultural and Engineering Sciences of the Federal University of Espírito Santo (CCAE-UFES). Chemical composition analyses of the oils were carried out in the Applied Chemistry laboratory of the Federal Institute of Espírito Santo (IFES, Alegre, ES).

Extraction and characterization of essential oils

EOs were extracted from fresh (FL) and dried CAT and MYR leaves[at room temperature (RT), 40 °C (T40 °C), and 60 °C (T60 °C) in a forced air circulation oven until constant mass (Otieno, Kariuki, & Wanjohi, 2020)] by hydrodistillation using a Clevenger apparatus, following the methodology recommended by the Brazilian Pharmacopoeia for volatile oils (Da Silva et al., 2019). Samples (approximately 80 g of leaves, in triplicates) and approximately 1.5 L of reverse osmosis water were placed in a 2 L round-bottom flask, which was then attached to the apparatus. The oil was extracted for 4 h after the water boiled. Subsequently, the obtained hydrosol was centrifuged at 14,000 rpm for 4 min to promote the separation between the aqueous and oily phases. With the aid of a Pasteur pipette, the oil (supernatant) was removed and stored in microtubes at -4 °C, protected from light.

The yield of the essential oil was expressed as % mass/mass, i.e., grams (g) of essential oil per gram of leaves, weighed on an analytical balance (precision of 0.0001 g), following the methodology described by Freitas, Martins and Vieira (2004). For quantifying the chemical constituents, EO samples were analyzed by Gas Chromatography with a Flame Ionization Detector (GC-FID) (Shimadzu GC-2010 Plus). For qualifying these chemical constituents, we used Gas Chromatography coupled with Mass Spectrometry (GC-MS) (Shimadzu GCMS-QP2010 SE). The protocol for quantifying and qualifying the chemical constituents of the EOs was carried out according to Dutra et al. (2020). Compounds with relative areas above 10% were considered major constituents.

Phytotoxicity analysis

An emulsion was prepared for each of the extracted oils using an ultrasound apparatus by mixing a solution of 100 mL containing 1g of oil and 1g of the Tween 80® surfactant, at a frequency of 60 Hz for 4 min, at intervals of 60 s. The emulsion was left to rest for approximately 7 days for homogeneity evaluation. After confirming the stability of the emulsions, the following concentrations of EO were prepared: 3000, 1500, 750, 375, and 187.5 μg mL^{-1}, and a solution of distilled water and Tween 80® was used as a negative control (C-).

For germination and root and shoot length assays, 2.5 mL of each diluted emulsion (including controls) was placed in Petri dishes (9 cm in diameter) containing filter paper and seeds of *L. sativa* variety Elba and *S. bicolor* variety Santa Elisa obtained from local agricultural houses. The experimental design was completely randomized with five repetitions per treatment, each repetition consisting of 25 seeds. The plates were sealed with plastic film and placed in a germination chamber (BOD - Biochemical oxygen demand) at 24 ± 2 °C and 16 h photoperiod.

Germination was evaluated at 8, 16, 24, 32, 40, and 48 h after the initial exposure to treatments. The number of germinated seeds was counted in each Petri dish and compared with the values observed in the control treatment to determine the germination speed index (GSI). The GSI was obtained according to the Equation 1:

$$GSI = (N8h \times 1) + (N16h - N8h) \times 1/2 + (N24h - N16h) \times 1/4 + + (N32h - N24h) \times 1/8 + (N40h - N32h) \times 1/16 + + (N48h - N40h) \times 1/32 \quad (1)$$

Where N x h represents the number of germinated seeds in a certain period of hours. Thus, after 48 h of exposure, the germination percentage (%G) was obtained considering germinated seeds as those that emitted a radicle protrusion of about 2 mm, and the GSI. The plates were kept in the BOD for 120 h, when root (root length – RL) and shoot (shoot length – SL) growth were evaluated using a digital caliper.

Statistical analysis

EO yield data were subjected to analysis of variance (ANOVA) followed by Tukey's test (P≤ 0.05). The variables evaluated in the phytotoxicity test were subjected to a triple factor analysis and the Dunnet test at 5% probability. All analyses were performed using R software.

Results and Discussion

Yield and chemical composition of EOs from *P. myrtoides* and *P. cattleyanum*

The interaction between species and temperatures was not significant (Table 1), thus allowing independent evaluation of the factors. The oils obtained from the leaves of MYR and CAT dried at 40°C showed the highest yield (0.86% and 1.07%, respectively), whereas the ones from fresh leaves had the lowest yield for both species (0.18% and 0.39%, respectively) (Figure 1).

This is in line with the results obtained by Júnior et al. (2020), who observed the highest yield using drying at 45°C for *Alpinia zerumbet*. In contrast, Schindler, Silva and Heinzmann (2018) observed that drying at ambient temperature was the most recommended for the extraction of EO from *Piper gaudichaudianum*. Drying treatments provide different oil yields due to the relationship with the volatilization temperature of their components, which are mainly located in glandular hairs, trichomes, and epidermal glands (Júnior et al., 2020).

Table 1: Analysis of variance of the essential oil yield of *P. myrtoides* and *P. cattleyanum* and their different leaf drying temperatures.

Source of variation	df	F Statistic	P-value
Species (A)	1	17.091	0.000779*
Leaf drying temperature (B)	3	60.178	<2.2e-16*
A X B	3	2.537	0.093286ns
Coefficient of variation	13.34%		

df: Degrees of freedom. Number of repetitions (n): 5. ns: not significant. * Significant at the 0.1% probability level (P ≤ 0.001).

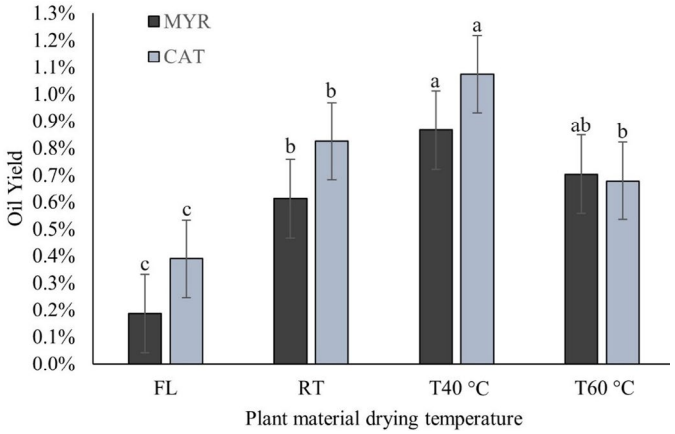

Figure 1: Essential oil yield of *P. myrtoides* (MYR) and *P. cattleyanum* (CAT) in the different pre-extraction treatments. Data are means ± standard deviation (n = 15). Significant differences among treatments (Tukey, p < 0.05) are shown by letters.

According to Júnior et al. (2020), besides preventing enzymatic degradation of the plant material, reducing the amount of water during drying increases the number of active principles per dry mass. Depending on the drying time and temperature used in different drying methods, oil yields can increase or decrease (Caputo et al., 2022). Thus, the quantity and composition of EOs are influenced by temperature during the drying process, explaining the low yield of oils extracted from fresh leaves of both MYR and CAT.

The compounds present in EOs are heat-sensitive substances, therefore, increasing the temperature during the drying of plant material can volatilize these compounds (Borges et al., 2020; Governici et al., 2020), and reduce the yield as observed for the CAT F60 oil when compared to lower temperatures (Figure 1).

Between 10 and 13 compounds were identified in the EOs (Table 2 and attachments), ranging from monoterpenes to sesquiterpenes classified into oxygenated and hydrocarbon categories. β-caryophyllene was the major compound (highest relative area) in all MYR and CAT EOs, except for the RT oil of MYR, whose major compound was α -bisabolol (14.46%) (Table 2). The concentration of β-caryophyllene varied between 10.84% and 23.6%, and the oils that presented higher concentrations of this sesquiterpene hydrocarbon were CAT essential oils, implying differences in the biological effect of each of these oils (Table 2).

Similar to yield, the chemical composition of EOs can change depending on the drying temperature, as enzymatic and non-enzymatic reactions during the drying of fresh materials can modify their phytochemical composition, reflecting on the biological activity and final quality of the oil (Caputo et al., 2022). Results obtained by Beigi, Torki-Harchegani and Ghasemi Pirbalouti (2018) showed that in certain situations, different temperatures during drying drastically alter the chemical profiles of EOs. Some compounds may increase or be lost due to the formation of new constituents by oxidation, glycoside hydrolysis, and esterification processes. Thus, the chemical composition of EOs can provide clues about their phytotoxic effects (Vasconcelos et al., 2021).

Table 2: Major chemical components identified in the essential oils of *P. myrtoides* and *P. cattleyanum*, extracted from fresh leaves (FL), leaves dried at room temperature (RT), leaves dried in an oven at 40 °C (T40 °C), and leaves dried in an oven 60 °C (T60 °C) using Rtx®-5MS column.

Components[a]	FL				RT				T40°C				T60°C				$RI_{calculat}$[e]	$RI_{tabulate}$[d]	Classification[f]
	MYR		CAT		MYR		CAT		MYR		CAT		MYR		CAT				
	$A_{relative}$ (%)[c]	$t_{retencion}$ (min.)[b]	$A_{relative}$ (%)[c]	$t_{retencion}$ (min.)[b]	$A_{relative}$ (%)[c]	$t_{retencion}$ (min.)[b]	$A_{relative}$ (%)[c]	$t_{retencion}$ (min.)[b]	$A_{relative}$ (%)[c]	$t_{retencion}$ (min.)[b]	$A_{relative}$ (%)[c]	$t_{retencion}$ (min.)[b]	$A_{relative}$ (%)[c]	$t_{retencion}$ (min.)[b]	$A_{relative}$ (%)[c]	$t_{retencion}$ (min.)[b]			
α-pinene	-	-	4.7	9227	4.08	9.43	9.8	9.24	-	-	5.5	9.23	-	-	7.7	9.24	929	932	MH
limonene	-	-	3.3	13.56	-	-	3.6	13.56	-	-	3.6	13.56	-	-	3.0	13.56	1025	1024	MH
1,8-cineole	-	-	17.4	13.69	-	-	10.2	13.68	-	-	19.5	13.69	-	-	6.8	13.68	1027	1026	MO
γ-terpinene	-	-	2.5	15.03	-	-	2.3	15.03	-	-	2.6	15.03	-	-	-	-	1056	1054	MO
α-terpineol	-	-	3.5	21.31	-	-	-	-	-	-	3.7	21.3	-	-	2.0	21.3	1188	1186	MO
α-copaene	3.36	31.16	3.8	29.54	2.42	28.38	5	29.54	3.02	28.38	4.2	29.53	2.84	31.16	5.9	29.54	1371	1374	SH
β-caryophyllene	12.71	31.82	22.6	31.4	11.87	31.81	13.5	31.38	12.31	31.82	23.6	31.38	10.84	31.82	18.7	31.39	1415	1417	SH
α-humulene	10.65	32.31	5.6	32.77	10.53	32.30	7.3	32.77	10.07	32.31	6.4	32.76	9.86	32.31	8.4	32.78	1448	1452	SH
α-farnesene	-	-	3.6	34.64	-	-	11.7	34.66	-	-	5	34.63	-	-	12.0	34.66	1494	1505	SH
δ-cadinene	-	-	2.5	35.68	-	-	3.5	35.68	-	-	2.5	35.67	-	-	4.0	35.69	1520	1522	SH
caryophyllene oxide	3.76	39.22	-	-	3.03	39.22	2.1	37.94	6.09	39.22	-	-	3.61	39.22	-	-	1579	1582	SO
γ-eudesmol	-	-	7.2	39.84	-	-	4.9	39.82	-	-	4.1	39.81	-	-	5.5	39.83	1628	1630	SO
β-eudesmol	-	-	9.2	40.5	-	-	8.5	40.5	-	-	7.6	40.48	-	-	8.8	40.5	1646	1649	SO
α-eudesmol	-	-	9.6	40.63	-	-	8.9	40.62	-	-	7.7	40.6	-	-	9.2	40.62	1649	1652	SO
α-bisabolol	11.04	42.94	-	-	14.46	42.94	-	-	10.8	42.94	-	-	9.82	42.94	-	-	1739	1685	OS
juniper camphor	2.15	43.08	-	-	-	-	-	-	2.87	43.08	-	-	-	-	-	-	1752	1696	OS
δ-cadinol	2.47	40.08	-	-	2.99	40.08	-	-	2.38	40.08	-	-	2.25	40.08	-	-	1696	1639	OS
β-bisabolene	2.05	-	-	-	-	-	-	-	2.08	35.72	-	-	2.25	35.72	-	-	1546	1505	SH
δ-cadinene	3.47	35.93	-	-	3.08	35.92	-	-	3.47	35.93	-	-	3.48	35.93	-	-	1563	1522	SH
germacrene B	2.23	36.37	-	-	2.24	-	-	-	2.13	36.37	-	-	2.32	36.37	-	-	1596	1559	SH
nerolidol	2.6	38.98	-	-	3.44	-	-	-	2.58	38.98	-	-	2.34	38.98	-	-	1606	1561	OS

[a] Major compounds identified using the Rtx®-5MS column; [b] Retention time in minutes; [c] Compounds with relative areas >2%; [d] Tabulated retention index (Adams, 2007; Lemmon et al., 2011); [e] Retention index calculated from saturated n-alkanes (C7-C40); [f] Classification of terpene compounds: monoterpene hydrocarbon (HM), oxygenated monoterpene (OM), hydrocarbon sesquiterpene (HS), and oxygenated sesquiterpene (OS). "-" symbol indicates the absence of the compound.

Phytotoxicity analysis of the emulsion of essential oils from *P. myrtoides* and *P. cattleyanum*

The emulsions of CAT oils T40 °C and T60 °C at a concentration of 1500 μg mL^{-1}, T60 °C at a concentration of 187.5 μg mL^{-1}, and T40 °C at a concentration of 3000 were more phytotoxic to *L. sativa* than the same treatments using MYR emulsions (Table 3). Additionally, CAT FL and RT emulsions at a concentration of 3000 μg mL^{-1} were statistically different from MYR and the control, reducing lettuce germination. We observed no significant difference among MYR oil emulsions on lettuce germination at the tested concentrations. *S. bicolor* germination was reduced by CAT and MYR RT emulsions at a concentration of 3000 μg mL^{-1} (Table 4), and by CAT FL emulsion at a concentration of 3000 μg mL^{-1}. Furthermore, CAT FL emulsion at a concentration of 3000 μg mL^{-1} was phytotoxic compared to the same treatment with MYR. While MYR FL emulsion at a concentration of 375 μg mL^{-1} was phytotoxic compared to the same treatment with CAT.

The effect of concentrations of MYR and CAT EOs extracted at different drying temperatures on the germination of seeds and initial development of *L. sativa* and *S. bicolor* seedlings can be visualized in Figures 2 and 3. Inhibition of seed germination and seedling growth are secondary effects of physiological processes, indicating a phytotoxic effect of the EO due to the compounds present. Allelochemicals impact cellular respiration and inhibit photosynthesis, affecting parameters such as root and hypocotyl length (Cândido et al., 2021).

Table 3: Effect of emulsions of essential oils from *P. myrtoides* (MYR) and *P. cattleyanum* (CAT) extracted from fresh leaves (FL), leaves dried at room temperature (RT), leaves dried in an oven at 40 °C (T40 °C), and leaves dried in an oven 60 °C (T60 °C) on seed germination and seedling initial growth of *L. sativa*.

Concentration		%G		GSI		SL (mm)		RL (mm)	
	Temperature	MYR	CAT	MYR	CAT	MYR	CAT	MYR	CAT
3000	FL	91.2 Aa	4.8 Bb*	9.95 Aab	0.22 Bb*	6.7 Aa	4.76 Ba*	39.43 Aa*	2.60 Ba*
	RT	95.2 Aa	11.2 Bb*	9.48 Ab	0.49 Bb*	6.61 Aa	4.23 Ba*	29.00 Ab	2.04 Ba*
	40	96 Aa	76 Ba	10.64 Aab	3.54 Ba*	5.80 Aa	5.57 Aa	15.00 Ac	3.68 Ba*
	60	98.4 Aa	86.4 Aa	11.42 Aa	4.47 Ba*	7.64 Aa	5.71 Ba	34.14 Aab	6.63 Ba*
1500	FL	94.4 Aa	96.0 Aa	11.06 Aa	7.44 Ba*	6.76 Aab	7.69 Aa	40.74 Aa*	11.26 Ba*
	RT	94.4 Aa	83.2 Aab	10.53 Aa	4.78 Bb*	5.85 Ab	6.06 Aa	26.86 Ab	9.27 Ba*
	40	96 Aa	76 Bb	10.7 Aa	5.24 Bb*	6.03 Ab	6.44 Aa	15.91 Ac	7.54 Ba*
	60	96.8 Aa	81.6 Bab	10.56 Aa	5.72 Bab*	8.86 Aa	6.53 Ba	40.12 Aa*	11.39 Ba*
750	FL	95.2 Aa	95.2 Aa	11.17 Aa	8.00 Ba*	7.76 Ab	8.49 Aab	38.42 Aa*	13.32 Ba
	RT	93.6 Aa	90.4 Aa	10.98 Aa	7.34 Ba*	2.08 Bc*	6.07 Ab	7.35 Ac*	13.24 Aa
	40	97.6 Aa	88.0 Aa	10.67 Aa	7.13 Ba*	8.68 Aab	8.19 Aab	22.96 Ab	9.12 Ba*
	60	96.0 Aa	93.6 Aa	11.17 Aa	7.06 Ba*	10.46 Aa	8.91 Aa	27.59 A b	13.39 Ba
375	FL	96.8 Aa	94.4 Aa	11.42 Aa	8.14 Ba*	5.51 Bb	8.63 Aab	25.21 Aa	13.56 Ba
	RT	95.2 Aa	92.8 Aa	11.39 Aa	8.08 Ba*	6.21 Ab	6.75 Ab	7.29 Ab*	13.43 Aa
	40	96.0 Aa	88.8 Aa	10.17 Aa	7.66 Ba*	10.74 Aa	8.93 Aab	25.15 Aa	10.29 Ba*
	60	94.4 Aa	96.8 Aa	10.08 Aa	7.93 Ba*	11.8 Aa*	9.36 Ba	35.41 Aa	14.17 Ba
187.5	FL	95.2 Aa	97.6 Aa	11.05 Aab	8.73 Ba*	11.24 Ab*	8.32 Bab	46.12 Aa*	13.97 Ba
	RT	96.0 Aa	93.6 Aab	11.61 Aab	8.59 Ba*	12.88 Aab*	6.80 Bb	45.25 Aa*	14.18 Ba
	40	95.2 Aa	91.2 Aab	9.88 Ab	8.53 Ba*	14.76 Aa*	10.13 Ba	39.26 Aa*	11.22 Ba*
	60	97.6 Aa	77.6 Bc	11.73 Aa	7.75 Ba*	12.28 Aab*	9.60 Ba	28.74 Ab	13.10 Ba
Control*		96.00		10.88		8.18		24.12	

Data are mean (n=5). Significant differences among treatments (Tukey, $P < 0.05$) are shown by letters. Capital and lowercase letters compare species and temperature treatments, respectively. Means followed by the symbol "*" differed from the control (Dunnet test, $P < 0.05$). SL – Shoot length, RL – Root length, expressed in mm/seedling.

Table 4: Effect of emulsions of essential oils from *P. myrtoides* (MYR) and *P. cattleyanum* (CAT) extracted from fresh leaves (FL), leaves dried at room temperature (RT), leaves dried in an oven at 40 °C (T40 °C), and leaves dried in an oven 60 °C (T60 °C) on seed germination and seedling initial growth of *S. bicolor*.

Concentration		%G		GSI		SL (cm)		RL (cm)	
	Temperature	MYR	CAT	MYR	CAT	MYR	CAT	MYR	CAT
30000	FL	81.6 Aa	62.4 Bb*	5.676 Aab*	5.182 Ab*	7.532 Aa*	9.634 Aa*	20.996 Aa	15.466 Aa
	RT	65.6 Ab*	64.8 Ab*	4.786 Ab*	4.826 Ab*	8.092 Aa*	7.900 Aa*	13.626 Aa	13.046 Aa
	40	76.0 Aab	83.2 Aa	5.696 Bab	8.028 Aa	8.558 Aa*	10.002 Aa*	10.790 Aa	19.416 Aa
	60	84.0 Aa	89.6 Aa	6.174 Ba	8.722 Aa	6.572 Aa*	8.362 Aa*	8.664 Aa	5.746 Aa*
1500	FL	72.8 Ab	83.2 Aa	5.534 Bb*	6.748 Ab	9.178 Aa*	12.718 Aa	26.642 Aa	23.954 Aa
	RT	73.6 Ab	80.8 Aa	5.674 Bb*	6.780 Ab	11.908 Aa*	11.692 Aa*	22.970 Aa	14.842 Aab
	40	89.6 Aa	84.8 Aa	7.382 Aa	8.302 Aa	9.944 Aa*	12.894 Aa	9.452 Ab	25.450 Aa
	60	87.2 Aab	88.8 Aa	6.602 Bab	8.838 Aa	9.760 Aa*	12.920 Aa	8.564 Ab	5.452 Ab*
750	FL	81.6 Aa	86.4 Aa	6.002 Ba	7.236 Aa	11.080 Ba*	16.176 Aa	16.288 Aa	23.142 Aab
	RT	81.6 Aa	84.0 Aa	6.754 Aa	7.434 Aa	10.446 Aa*	13.160 Aa	7.984 Ba*	24.200 Aa
	40	87.2 Aa	85.6 Aa	6.330 Ba	7.860 Aa	10.764 Aa*	13.226 Aa	14.244 Aa	10.364 Abc
	60	89.6 Aa	82.4 Aa	6.552 Ba	7.550 Aa	11.432 Aa*	0 Bb*	12.440 Aa	0 Bc*
375	FL	72.8 Ba	93.6 Aa	5.940 Ba	7.614 Aa	12.750 Ba	19.370 Aa	19.578 Aa	26.194 Aa
	RT	82.4 Aa	86.4 Aa	6.754 Aa	7.526 Aa	17.340 Aa	17.074 Aa	16.852 Aa	22.842 Aa
	40	87.2 Aa	84.8 Aa	6.834 Ba	8.040 Aa	15.948 Aa	16.668 Aa	15.076 Aa	6.378 Ab*
	60	87.2 Aa	80.8 Aa	5.972 Ba	7.852 Aa	14.702 Aa	0 Bb*	16.462 Aa	0 Bb*
187.5	FL	84.8 Aa	84.8 Aa	6.152 Ba	7.270 Aa	17.400 Ab	16.176 Aa	20.986 Aab	28.424 Aa
	RT	91.2 Aa	85.6 Aa	6.684 Aa	7.046 Aa	26.378 Aa	13.160 Ba	17.676 Ab	24.244 Aa
	40	83.2 Aa	72.8 Aa	5.936 Ba	7.050 Aa	17.860 Ab	13.226 Aa	30.702 Aa	5.398 Bb*
	60	90.4 Aa	80.0 Aa	6.706 Aa	7.634 Aa	22.000 Aab	0 Bb*	13.544 Ab	0 Bb*
Control*		83.2		7.268		19.358		24.496	

Data are mean (n=5). Significant differences among treatments (Tukey, $P < 0.05$) are shown by letters. Capital and lowercase letters compare species and temperature treatments, respectively. Means followed by the symbol "*" differed from the control (Dunnet test, $P < 0.05$). SL – Shoot length, RL – Root length, expressed in cm/seedling.

Regarding the germination speed index (GSI) of *L. sativa*, all CAT emulsions tested were phytotoxic compared to the control and reduced GSI (Table 3). For *S. bicolor*, overall, all treatments using MYR were more phytotoxic than the ones using CAT oils but did not differ from the control, except for FL and RT emulsions at a concentration of 1500 μg mL^{-1}. FL and RT emulsions at 3000 μg mL^{-1} from CAT and MYR were phytotoxic compared to the control (Table 4).

The results obtained here corroborate with those of Vasconcelos et al. (2019), in which the EO of *P. myrtoides* and *P. cattleyanum* at concentrations of 3000 μg mL^{-1} were efficient in inhibiting the germination of *L. sativa* and *S. bicolor* seeds. Among the germination parameters, IVG is considered the most important because some allelopathic compounds do not influence final germination but cause a delay in GSI (Costa et al., 2020) The delay observed in radicle emergence may be related to the interference of allelochemicals in the reactivation of the mitochondrial cycle, oxidative phosphorylation, as well as protein synthesis from substrates (enzymes, ribosomes, and mRNA) that occur during germination phases I and II (Gindri et al., 2020).

The shoot length (SL) of *L. sativa* was reduced using CAT FL and RT emulsions at a concentration of 3000 μg mL^{-1}, as well as MYR RT emulsion at a concentration of 750 μg mL^{-1} (Table 3). For *S. bicolor*, SL was reduced in all CAT and MYR emulsions at a concentration of 3000 μg mL^{-1} (Table 4), regardless of the drying temperature used. Additionally, concentrations from 187.5 μg mL^{-1} to 750 μg mL^{-1} of CAT T60°C emulsion completely inhibited both aerial and root growth of sorghum (Figure 3). Differences in sensitivity between target species are commonly reported in studies investigating allelopathy and phytotoxicity of plants (Hazrati et al., 2017; Vasconcelos et al., 2019). These differences are related to variations in absorption mechanisms, translocation, and site of action of substances among different target species, as stated by Choudhary et al. (2023).

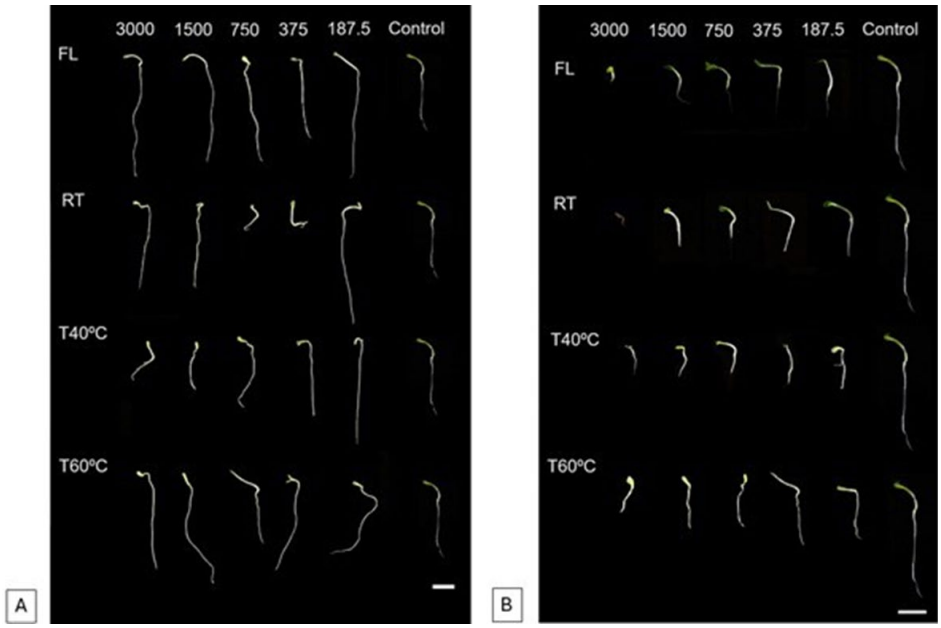

Figure 2: Seeds of *L. sativa* exposed to different concentrations (µg mL^{-1}) of the essential oil emulsion extracted from fresh leaves (FL), leaves dried at room temperature (RT), leaves dried in an oven at 40 °C (T40 °C), and leaves dried in an oven at 60 °C (T60 °C) of *Psidium myrtoides* (A) and *Psidium cattleyanum* (B). Control = distilled water and Tween 80®. Scale bar = 1 cm.

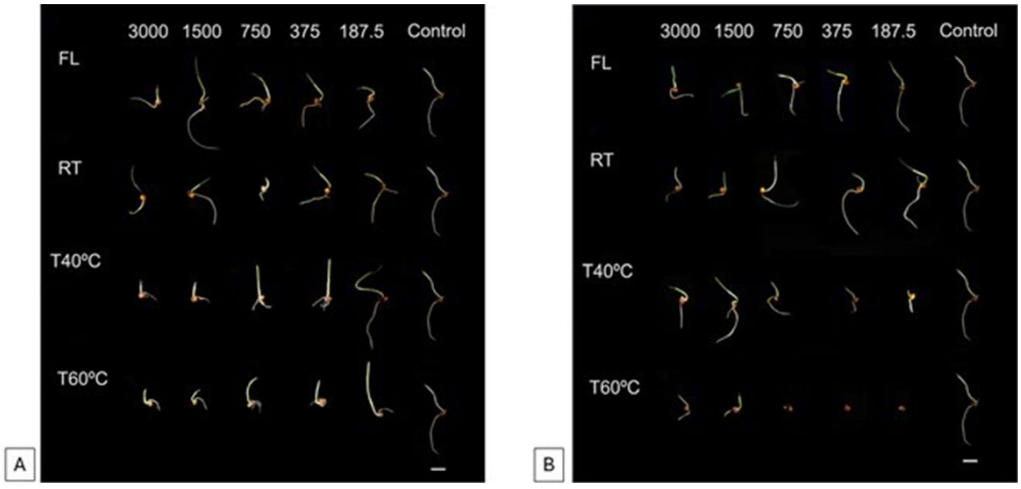

Figure 3: Seeds of *S. bicolor* exposed to different concentrations (µg mL^{-1}) of the essential oil emulsion extracted from fresh leaves (FL), leaves dried at room temperature (RT), leaves dried in an oven at 40 °C (T40 °C), and leaves dried in an oven at 60 °C (T60 °C) of *Psidium myrtoides* (A) and *Psidium cattleyanum* (B). Control = distilled water and Tween 80®. Scale bar = 1 cm.

CAT emulsions were mostly phytotoxic to *L. sativa* compared to MYR emulsions, and all treatments at concentrations of 1500 µg mL^{-1} and 3000 µg mL^{-1} differed from the control. All MYR emulsions at a concentration of 187.5 µg mL^{-1} acted as growth inducers for both aerial and root growth of lettuce. These effects can be evaluated and directed toward the production of biofertilizers (Choudhary et al., 2023). However, MYR RT emulsion at a concentration of 750 µg mL^{-1} reduced root length (RL) in both *L. sativa* and *S. bicolor*. Similar to lettuce, sorghum root growth was reduced in all CAT and MYR emulsions at a concentration of 3000 µg mL^{-1}, regardless of the drying temperature used.

EOs have strong herbicidal activity, and this phytotoxicity is attributed to the presence of 1,8-cineole and α-pinene (Abd-ElGawad et al., 2021). Among the observed effects are reductions in cell division, chlorophyll content, and cellular respiration (Cândido et al., 2021), which are reflected in reduced germination percentage and growth.

In addition to the reduction in RL, we observed abnormal seedlings with oxidized and necrotic roots. Seedling growth depends on DNA synthesis, mitotic divisions, and mobilization of seed reserves (Jhanji et al., 2024), which, when inhibited by allelochemicals, compromise their normal development. It is known that α-pinene can strongly inhibit mitochondrial ATP production in seedlings, inhibiting initial root growth and causing oxidative damage to root tissue (Zhou et al., 2021).

The phytotoxic effects of CAT and MYR essential EOs on *L. sativa* and *S. bicolor* can also be associated with the presence of β-caryophyllene, a sesquiterpene hydrocarbon (Vasconcelos et al., 2019), which has confirmed allelopathic effects. Sesquiterpenes affect plant growth through oxidative stress along with effects on physiological processes, such as mitochondrial respiration, microtubule distribution, and organization (Araniti et al., 2016).

Furthermore, EOs with a high content of monoterpenes are known for their ability to suppress weeds (Fagodia et al., 2017). For example, the hydrogenated monoterpenoid limonene, found in *P. guajava*, is known to block the nitrogen cycle and inhibit cytochrome respiration, seed germination, and growth in neighboring plants (Maffei, Gertsch, & Appendino, 2011). In this sense, the phytotoxic activity found in this study can be mainly attributed to the presence of sesquiterpenes and monoterpenes.

Conclusions

The highest yield of EOs from *P. cattleyanum* and *P. myrtoides* is obtained from leaves dried at 40°C. The chemical profile of EOs vary according to different drying temperatures, and the phytotoxicity effect of the oils can be explained by the synergy of the compounds present. The EO emulsion from leaves dried at room temperature of both species showed greater phytotoxic activity in the bioassays.

Author Contribution

Conceptual idea: Bergamin, A.S.; Vasconcelos, L.C.; Praça-Fontes, M.M.; Methodology design: Bergamin, A.S.; Vasconcelos, L.C.; Data collection: Mariano, G.F.; Izidio, I.S.; Data analysis and interpretation: Bergamin, A.S.; Vasconcelos, L.C.; Mendes, L.A.; Writing and editing: Bergamin, A.S.; Praça-Fontes, M.M.

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