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Scientific Article

Yield, composition and toxicity of piperaceae volatiles to pest insects

Rendimento, composição e toxicidade de voláteis de piperáceas para insetos -praga

Jamila F. Mendonça[1], Adalberto H. De Sousa[1]*, Lêda R. A. Faroni[2], Carromberth C. Fernandes[1], Ana C. V. dos Santos[1], Lucas M. Lopes[1], Maria S. S. Ferraz[2], Lucas H. F. Prates[2]

[1]Center for Biological and Nature Sciences, Universidade Federal do Acre, Rio Branco, AC, Brazil. [2]Department of Agricultural Engineering, Universidade Federal de Viçosa, Viçosa, MG, Brazil.

ABSTRACT - The objective of this study was to investigate the influence of leaf drying techniques (bench and oven at 35 and 45 °C) on the essential oil (EO) yield of *Piper aduncum* L., *Piper anonifolium* Kunth, *Piper crassinervium* Kunth and *Piper hispidinervum* C. DC., and to analyze the chemical profile of EOs and the insecticidal potential of these oils against *Ascia monuste orseis* (Godart), *Atta sexdens* L., *Zabrotes subfasciatus* (Boheman), *Cryptolestes ferrugineus* (Stephens) and *Sitophilus zeamais* Motschulsky. EO yield was evaluated using four replicates of 100g of dry leaves. The EOs were obtained by hydrodistillation and subjected to GC-MS analysis to assess the chemical composition. Concentrations of 2.60 and 157.25 nL/cm^2 were used in the oil toxicity bioassays. EO yield was higher in the species *P. aduncum* and *P. hispidinervum* using leaves dried in oven at 45 °C, with average yields of 4.72±0.04% and 2.61±0.26%, respectively. The major constituents present in the EOs of *P. hispidinervum* and *P. aduncum* were Safrole (98.80%) and Apiole (90.00%). For *P. anonifolium*, the major constituents were α-Muurolene (23.11%), γ-Muurolene (16.60%) and Cadina-1(10), while for *P. crassinervium*, they were Viridiflorol (27.70%) and Sabinene (15.50%). It was found that the EOs of *P. aduncum*, *P. anonifolium*, *P. crassinervium* and *P. hispidinervum* had a toxic effect on insects, except for *P. anonifolium* and *P. crassinervium* for *S. zeamais*. EO yield was higher in the species *P. aduncum* and *P. hispidinervum*, and these oils caused a higher mortality rate for the investigated insects.

Keywords: *Piper*. Essential oils. Secondary metabolites. Major compounds. Bioinsecticide.

RESUMO - O objetivo desta pesquisa foi investigar a influência de técnicas de secagem de folhas (bancada e em estufa a 35 e 45 °C) sobre o rendimento do óleo essencial (OE) de *Piper aduncum* L., *Piper anonifolium* Kunth, *Piper crassinervium* Kunth e *Piper hispidinervum* C. DC.; analisar o perfil químico dos OEs; e o potencial inseticida destes óleos para *Ascia monuste orseis* (Godart), *Atta sexdens* L., e para *Zabrotes subfasciatus* (Boheman), para o besouro *Cryptolestes ferrugineus* (Stephens) e para *Sitophilus zeamais* Motschulsky. Avaliou-se o rendimento do OE utilizando quatro repetições de 100g de folhas secas. Os OEs foram obtidos por hidrodestilação e submetidos à análise por CG-EM para a constatação da composição química. Utilizou-se as concentrações 2,60 e 157,25 nL/cm^2 nos bioensaios de toxicidade dos óleos. O rendimento dos OEs foi maior nas espécies *P. aduncum* e *P. hispidinervum* utilizando folhas secas em estufa a 45 °C, com rendimentos médios de 4,72±0,04% e 2,61±0,26% respectivamente. Os constituintes majoritários presentes nos OEs de *P. hispidinervum* e *P. aduncum,* foram o Safrol (98,80%) e Apiole (90,00%). Para *P. anonifolium,* foram o *α*-Muuroleno (23,11%), *γ*-Muuroleno (16,60%) e Cadina-1(10), enquanto para *P. crassinervium,* foram o Viridiflorol (27,70%) e Sabineno (15,50%). Constatou-se que os OEs de *P. aduncum*, *P. anonifolium*, *P. crassinervium* e *P. hispidinervum* apresentaram efeito tóxico para os insetos, exceto *P. anonifolium* e *P. crassinervium* para *S. zeamais*. O rendimento dos OEs foi maior nas espécies *P. aduncum* e *P. hispidinervum* e estes óleos causaram maior taxa de mortalidade para os insetos investigados.

Palavras-chave: *Piper*. Óleos essenciais. Metabólitos secundários. Compostos majoritários. Bioinseticida.

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***Corresponding author:**
<adalberto.sousa@ufac.br>

INTRODUCTION

Essential oils (EOs) are important raw materials for the agronomic, pharmaceutical and cosmetic industries. Formed by terpene, volatile and liquid substances, originating from the secondary metabolism of plants, EOs derive from terpenoids originating from mevalonic acid, or from phenylpropanoids, from shikimic acid, and usually one of them will be the predominant. The quantity of these constituents directly affects the quality of EOs (DHIFI et al., 2016). EOs have low concentrations in the secretory structures, yield and chemical composition that vary among the species, and is influenced by seasonality, plant age, time of collection and drying of the botanical material (SCHINDLER; SILVA; HEINZMANN, 2018).

In oilseed species, the material needs to be dried after collection to avoid the development of microorganisms that decompose and modify the aromatic principles. Drying reduces the time and cost of distillation, stabilizes the color, aroma and texture, ruptures glandular walls and secretory structures, causing leakage of the chemical compounds present inside, thus contributing to increasing EO yield (PEREIRA et al., 2013).

Several botanical families produce EOs with insecticidal potential, such as Piperaceae, which is known to exhibit species with high EO content. In Brazil it is represented by the genera *Manekia*, *Peperomia* and *Piper*, the last of which being the one with the greatest diversity of species. In Piperaceae species, EOs are mainly made up of terpenes, phenylpropanoids, aldehydes, hydrocarbons, and ketones. These act as allelochemicals, against pathogenic and signaling agents for attraction and defense against herbivores, and cause toxic effects on insects (RUIZ-VÁSQUEZ et al., 2022).

Piper species have been investigated as promising sources of secondary metabolites and chemical compounds such as amides, terpenes, benzoic acid, carotenoids, lignans, and alkaloids, which have significant phytopharmaceutical effects, including allelopathic/phytotoxic, antifungal, insecticidal and disruptor of pest insect development, besides having an ovicidal, nematicidal, and antifeedant effect (RUIZ-VÁSQUEZ et al., 2022).

The demand for synthetic chemical insecticides is primarily concentrated in the agriculture and forestry sectors. In forest plantations, the main pest is leaf-cutting ants, such as *Atta* ('saúvas') and *Acromyrmex* ('quenquéns'), as they cut and transport part of the plants to the anthill for growing of fungi, the colony's food base. Depending on the level of attack, the establishment of these plantations becomes unfeasible (ZANETTI et al., 2014).

In horticulture, the damage caused by *Ascia monuste orseis* Godart (Lepidoptera: Pieridae), an important pest of Brassicaceae, stands out. The caterpillars defoliate the plant and can cause total loss of production (MAPELI et al., 2015). In the agricultural storage sector, the maize weevil *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae) and the beetle *Cryptolestes ferrugineus* Stephens (Coleoptera: Laemophloeidae), among others, stand out, attacking cereal grains and by-products, as well as the bean weevil *Zabrotes subfasciatus* Boheman (Coleoptera: Chrysomelidae) in legume grains. An alternative source is botanical insecticides, which can be used in the form of EOs, extracts, and powder.

The objectives of this study were to investigate the influence of leaf drying techniques (bench and oven at 35 and 45 °C) on the EO yield of *Piper hispidinervum* C. DC., *Piper aduncum* L., *Piper anonifolium* Kunth and *Piper crassinervium* Kunth, to analyze the chemical profile of Eos, and to evaluate their insecticidal potentials against *Ascia monuste orseis*, *Atta sexdens* L. (Hymenoptera: Formicidae), *Cryptolestes ferrugineus*, *Zabrotes subfasciatus* and *Sitophilus zeamais*.

MATERIAL AND METHODS

Essential oil extraction and yield

Leaves of *Piper hispidinervum*, *P. aduncum*, *P. anonifolium* and *P. crassinervium* were collected in the morning in the municipalities of Bujari and Rio Branco, Acre, Brazil, with the following geographic coordinates: (9° 42'17.26''S, 68°3'15.63''W; 9°57'9.24''S, 67°50'27.11''W; 10°04'09.2''S, 67°36'31.3''W and 9°42'17.26''S, 68° 3'15.63''W), respectively. The climate of the collection regions is humid equatorial.

The specimens were deposited in the UFACPZ Herbarium of the Federal University of Acre, under the registration numbers: UFACPZ 20.647, UFACPZ 20.646, UFACPZ 20.611 and UFACPZ 20.657, respectively. The species were identified by PhD Elsie Franklin Guimarães, from the Herbarium of the Botanical Garden of Rio de Janeiro (RB Herbarium).

The leaves were dried using the techniques of bench and air circulation and renewal oven (SL-102), at temperatures of 35 °C and 45 °C, until reaching constant weight. For the extraction of the EOs, four replicates of 100g of dry leaves were subjected to hydrodistillation for 4 hours in a simple Clevenger device, 5 L volumetric flask and heating mantle (0321A28, Quimis, Brazil). The EOs were separated from the hydrosol by decantation in a separation funnel. The oils were dried with anhydrous sodium sulfate (Synth, 99.0%, Brazil), and stored in amber bottles at a temperature of 4±1 ° C. EO yield was expressed as a percentage, calculated by Equation 1, adapted from Silva et al. (2013).

$$Y\% = \frac{V(mL) * D(g/mL)}{M\ (g)} \qquad (1)$$

Where: Y% = Yield (%); V = Oil volume (mL); D = Mass of 1mL of oil (g); M = Dry mass of leaves (g).

Composition of essential oils

Chromatographic analysis of the oils extracted at 35 °C was carried out at the Federal University of Viçosa-UFV, in the Chemistry Laboratory. For this analysis, Chromasolv® acetonitrile, ≥99.9%, from Sigma-Aldrich (St. Louis, MO, USA) was used as solvent. The EOs were diluted in acetonitrile at 50 µL/L and analyzed by Gas Chromatography coupled to Mass Spectrometry (GC-MS) (GC7820A-5977B, Agilent, United States of America) to identify their constituents. A standard solution of C7-C30 Alkane at 1000 µg/mL in Hexane (Sigma Aldrich, St. Louis, MO, USA) was injected for calculating the retention index and confirming the compounds identified by GC-MS (ADAMS, 2007).

The GC-MS was operated in full scan mode (mass acquisition range m/z 50-450), using 70 eV ionization energy. The gas chromatograph was operated at a division ratio of 20:1 with an injector temperature of 220 °C. The initial temperature of the column furnace was set to 60 °C, with a heating rate of 2 °C/min up to 200 °C, followed by an increase in the heating rate from 5 °C/min up to 250 °C. Helium was used as a carrier gas, with a column flow rate of 1.2 mL/min. The total data acquisition time was 80 min. A 1 µl sample was injected by the AOC-20i auto injector (Agilent, United States of America) into the chromatograph. The separations were performed in a 30 m x 0.25 mm internal diameter x 0.25 µm HP-5 ms capillary column (Agilent Technologies, Palo Alto, CA, USA) with a stationary phase of 5% Diphenyl/95% Polydimethylsiloxane.

Obtaining and rearing insects

Eggs of *A. monuste orseis* were collected from kale plants (*Brassica oleracea* var. *acephala*), kept in 300 mL flasks under constant conditions of temperature (25±2 °C) and

relative humidity (70±5%). After hatching, the larvae were fed daily and, on the third day, used in the bioassays (MAPELI et al., 2015). The leaf-cutting ants *A. sexdens* L. were collected manually on the UFAC campus, in a stable nest and stored in a 1-L glass jar until they were used in the bioassays, which were carried out on the same day of collection (JUNG et al., 2013). The weevils *C. ferrugineus*, *S. zeamais* and *Z. subfasciatus* were reared in 1-L glass jars, under conditions of ambient temperature (25±2 °C) and relative humidity (70±5%). Whole grains of maize were used in the rearing of *S. zeamais*, crushed grains of maize for *C. ferrugineus* and intact grains of beans in the rearing of *Z. subfasciatus*. Grains with moisture content of 13%, wet basis (w.b.), previously fumigated with phosphine (PH_3) and refrigerated, avoiding re-infestation.

Mortality bioassays

Mortality bioassays were performed in a BOD incubator under constant conditions of temperature (25±2 °C) and relative humidity (70±5%). These were performed in Petri dishes (90 × 15 mm) with the bottom covered with filter paper and edges coated with Teflon® PTFE (DuPont, São Paulo, Brazil). Five *A. monuste orseis* larvae were used in each experimental unit, with seven replicates. For *A. sexdens*, ten insects with ten replicates were used. For *C. ferrugineus* and *Z. subfasciatus*, 25 insects were used, with four replicates, and for *S. zeamais*, 50 insects were used, with four replicates. In each experimental unit, 1 mL of EOs diluted in Propanone (Synth, 99.5%, Brazil) was applied at concentrations of 2.6 and 157.25 nL/cm^2. In the control, 1 mL of Propanone was used, and the Petri dishes were kept in an ambient room for 5 min for solvent evaporation. After this period, the insects were placed on the Petri dishes, and the mortality rate was calculated after 24 hours of exposure.

Statistical analysis

A completely randomized design in a 3 × 4 factorial scheme was used for determining the yield of the Eos; the first factor corresponds to the leaf drying techniques and the second factor to the Piperaceae species. For the toxicity of the EOs, the factorial scheme used was 2 × 4, in which the first factor corresponds to the EO concentrations and the second factor to the four Piperaceae species. The data were subjected to analysis of variance (ANOVA), and the means were compared by Tukey test ($P \leq 0.05$), using Sisvar 5.6 software. Graphs were constructed using SigmaPlot 11 software (StatSoft, Inc., Tulsa, OK, USA).

RESULTS AND DISCUSSION

Essential oil yield

EO yield varied significantly between the species ($F_{3;36}$=498.60; P≤0.0001), between drying conditions ($F_{2;36}$=16.28; P≤0.0001) and there was an interaction between these two factors ($F_{6;36}$=7.93; P≤0.0001). Table 1 shows that the yield of *P. aduncum* and *P. hispidinervum* EOs was significantly higher (P≤0.01) with leaves dried in oven at 45 ° C, with yields of 4.72±0.04% and 2.61±0.26%, respectively (Table 1). The yield of *P. aduncum* EO was 44.71% higher than that of *P. hispidinervum* under this drying condition. The yield was significantly lower for *P. anonifolium* and *P. crassinervium* (P≤0.01) and there was no significant difference between these Piperaceae species (Table 1).

Table 1. Yield of essential oils of *Piper aduncum*, *P. anonifolium*, *P. crassinervium* and *P. hispidinervum*.

Botanical species	Yield (%) (±M.S.E.)		
	Bench	35 °C	45 °C
Piper aduncum	3.53±0.25 Ab*	3.36±0.13 Ab	4.72±0.04 Aa
P. anonifolium	0.13±0.01 Ca	0.20±0.01 Ca	0.12±0.01 Ca
P. crassinervium	0.32±0.02 Ca	0.28±0.04 Ca	0.22±0.01 Ca
P. hispidinervum	2.00±0.09 Bb	1.76±0.08 Bb	2.61±0.26 Ba

(*) Means followed by the same uppercase letter in the column and lowercase letter in the row did not differ statistically from each other by Tukey test (P<0.05); M.S.E. = Mean standard error.

The EO yield of *P. hispidinervum* corroborates with the oil yield of this species (2.6%) obtained from leaves collected in Porto Alegre, RS, Brazil, and dried at temperature of 40 °C (ROSSA et al., 2018). As for *P. aduncum*, a lower oil yield (1.3%) was observed with leaves collected in Brasília, DF, Brazil, and dried at a temperature of 38 °C (POTZERNHEIM et al., 2012). On the other hand, the EOs of *P. anonifolium* and *P. crassinervium* obtained in other Amazonian regions showed low yield (0.1 - 0.6%), coinciding with the result obtained (LUZ; ZOGHBI; MAIA, 2003; SILVA et al., 2014). Variations in EO yields are common and may occur due to the plant's metabolic pathway, age, seasonality, period and time of collection, as well as the occurrence of predators and pathogens (SCHINDLER; SILVA; HEINZMANN, 2018). In the present study, a variation in the EO yields of the studied species was observed. *P. aduncum* showed the highest yield, followed by *P. hispidinervum*, *P. crassinervium* and *P. anonifolium*. This result may be related to intrinsic and extrinsic factors to the plant, since the specimens collected, in addition to being of different species, showed varied vegetative stages.

Composition of essential oils

Hydrodistillation of the leaves produced pale yellow to dark yellow EOs. The studied species showed high chemical variation in their EOs. GC-MS analysis allowed the identification of 63 chemical compounds in the EOs of *P.*

aduncum, *P. anonifolium*, *P. crassinervium* and *P. hispidinervum* species (Table 2). The EOs had monoterpenes, sesquiterpenes and diterpenes in their composition. The dominant chemical classes in *P. aduncum* and *P. hispidinervum* were the phenylpropanoids Apiole and Safrole (Figures 1A and 1B) with 90% and 98.8% of the chemical composition, respectively (Table 2). In *P. anonifolium* and *P. crassinervium*, the sesquiterpene class dominated, with percentages of 100% and 80%, respectively (Table 2).

The EO of *P. aduncum* contained 13 compounds, with the phenylpropanoid Apiole (90.00%) being the major constituent (Table 2, Figure 2A). There are few reports of this compound as the majority in the EO of this species; Dillapiole is commonly found. Santana et al. (2015) reported the presence of Apiole (28.60%) in the EO from leaves of this species collected in the state of Rondônia, Brazil. On the other hand, in leaves from the state of Amazonas, Brazil, Apiole constituted 0.38% of the composition and Dillapiole stood out with 86% (SILVA et al., 2013).

Table 2. Percentage of the compounds of the EOs of leaves (dried at 35 °C) of *Piper aduncum* (P.ad.), *P. anonifolium* (P.an), *P. crassinervium* (P.cr) and *P. hispidinervum* (P.hi).

			---------- Relative area (%)[2] ----------			
Compound	Class	RT[1] (min.)	P.ad	P.an	P.cr	P.hi
α-Pinene	Monoterpene	6.551	–	–	5.64	–
Sabinene	Monoterpene	8.307	–	–	15.49	–
Limonene	Monoterpene	10.237	–	–	0.95	–
α-Terpineol	Monoterpene	19.011	–	–	0.34	–
Safrole	Phenylpropane	25.113	–	–	–	98.83
δ-Elemene	Sesquiterpene	27.842	–	–	0.40	–
α-Cubebene	Sesquiterpene	28.572	–	1.61	1.33	–
Cyclosativene	Sesquiterpene	29.424	–	5.33	–	–
α-Copaene	Sesquiterpene	30.120	0.36	–	0.66	–
β-Elemene	Sesquiterpene	31.180	0.28	–	7.07	–
α-Gurjunene	Sesquiterpene	32.145	–	–	2.96	–
Caryophillene (Z)	Sesquiterpene	32.710	2.12	5.62	3.36	–
β-Copaene	Sesquiterpene	33.318	–	–	0.57	–
γ-Elemene	Sesquiterpene	33.666	–	–	0.40	–
α-Guaiene	Sesquiterpene	34.635	–	1.39	–	–
α-Humulene	Sesquiterpene	34.744	0.44	–	–	–
β-Humulene	Sesquiterpene	34.752	–	0.46	0.88	–
Alloaromadendrene	Sesquiterpene	34.935	–	0.48	–	–
Isocaryophillene	Sesquiterpene	35.178	–	–	2.22	–
Ishwarane	Sesquiterpene	35.222	–	4.89	–	–
γ-Muurolene	Sesquiterpene	36.265	–	16.60	1.01	–
Germacrene D	Sesquiterpene	36.413	1.62	–	2.61	–
β-Selinene	Sesquiterpene	36.686	–	–	1.22	–
Valencene	Sesquiterpene	36.712	–	3.24	–	–
Ledene	Sesquiterpene	37.251	–	–	2.09	–
Bicyclogemacrene	Sesquiterpene	37.329	0.53	–	–	1.17
β-Curcumene	Sesquiterpene	37.481	–	2.45	–	–
α-Muurolene	Sesquiterpene	37.712	–	23.11	0.74	–
Pentadecane	Sesquiterpene	37.764	0.61	–	–	–
γ-Gurjunene	Sesquiterpene	37.808	–	–	0.68	–
β-Bisabolene	Sesquiterpene	38.207	–	1.87	–	–
γ-Cadinene	Sesquiterpene	38.386	–	–	0.71	–
δ-Cadinene	Sesquiterpene	38.977	–	–	2.00	–
Myristicin	Phenylpropane	38.998	1.70	–	–	–
Cadina-1(10),4-diene	Sesquiterpene	39.020	–	11.20	–	–

[1]RT = Retention time on the HP-5MS (30m × 0.25mm ID × 0.25µm) column; [2]Expressed as area (%).

Table 2. Continuation.

			---------- Relative area (%)[2] ----------			
Compound	Class	RT[1] (min.)	P.ad	P.an	P.cr	P.hi
α-Calacorene	Sesquiterpene	40.050	–	0.65	–	–
Germacrene B	Sesquiterpene	40.754	–	–	0.43	–
Dillapiole	Phenylpropane	40.810	0.57	–	–	–
Nerolidol (E)	Sesquiterpene	41.454	–	–	4.75	–
Spathulenol	Sesquiterpene	42.006	0.35	–	0.49	–
Caryophyllene oxide	Sesquiterpene	42.266	0.35	–	–	–
Globulol	Sesquiterpene	42.345	–	–	2.10	–
Guaiol	Sesquiterpene	42.770	1.09	–	–	–
Viridiflorol	Sesquiterpene	42.845	–	–	27.74	–
Cedrol	Sesquiterpene	44.735	–	2.53	–	–
Cubenol	Sesquiterpene	44.857	–	–	1.43	–
Muurola-4,10(14)-dien-1β-ol	Sesquiterpene	44.913	–	7.28	–	–
Epicubenol	Sesquiterpene	45.074	–	0.94	–	–
Apiole	Phenylpropane	45.122	90.00	–	–	–
Torreyol	Sesquiterpene	45.904	–	4.10	0.92	–
Epi-α-cadinol	Sesquiterpene	46.308	–	5.16	2.59	–
α-Cadinol	Sesquiterpene	46.326	–	–	5.35	–
Himachalol	Phenylpropane	47.108	–	–	0.41	–
β-Copaen-4α-ol	Sesquiterpene	47.125	–	0.58	–	–
Cembrene	Diterpene	47.347	–	–	0.45	–
Isolongifolan-7α-ol	Sesquiterpene	47.599	–	0.26	–	–
Ylangenol	Sesquiterpene	49.250	–	0.24	–	–
Monoterpenes					14%	
Sesquiterpenes			77%	100%	80%	50%
Phenylpropanoids			23%		3%	50%
Total			100%	100%	97%	100%

[1]RT = Retention time on the HP-5MS (30m × 0.25mm ID × 0.25µm) column; [2]Expressed as area (%).

In *P. anonifolium*, 26 constituents were identified (Table 2, Figure 2B), with the sesquiterpene α-Muurolene (23.00%) (Figure 1C) being the major compound, followed by γ-Muurolene (16.60%) (Figure 1D) and Cadina-1(10),4-Diene (11.00%) (Figure 1E). The production of these constituents may be related to the protection of the plant due to the attack of microorganisms. In EOs obtained from leaves of this species, collected in Amazonian regions, the constituents Selin-11-en-4-*α*-ol (20.00%), *β*-Selinene (12.70%), *α*-Selinene (11.90%) were identified (SILVA et al., 2014), as well as Caryophyllene (11.30%), Germacrene-D (9.60%), *α*-humulene (6.60%), *δ*-cadinene (6.60%) and (-)-*β*-copaene (5.80%) (RUIZ-VÁSQUEZ et al., 2022).

The EO of *P. crassinervium* showed 35 compounds (Table 2, Figure 2C), with the sesquiterpene Viridiflorol (27.70%) (Figure 1F) being the major constituent, followed by the monoterpene Sabinene (15.50%) (Figure 1G) and the sesquiterpene β-Elemene (7.00%) (Figure 1H). Other constituents were reported as the main ones in the composition of EO of this species collected in the state of Acre, Brazil, such as *β*-Caryophyllene (17.70%), *γ*-Elemene (14.40%) and *β*-Elemene (10.90%) (LUZ; ZOGHBI; MAIA, 2003). On the other hand, the EO of leaves collected in the state of São Paulo, Brazil, had Germacrene (D) (14.00%) and Spathulenol (9.68%) as main constituents (MORANDIM-GIANNETTI et al., 2010).

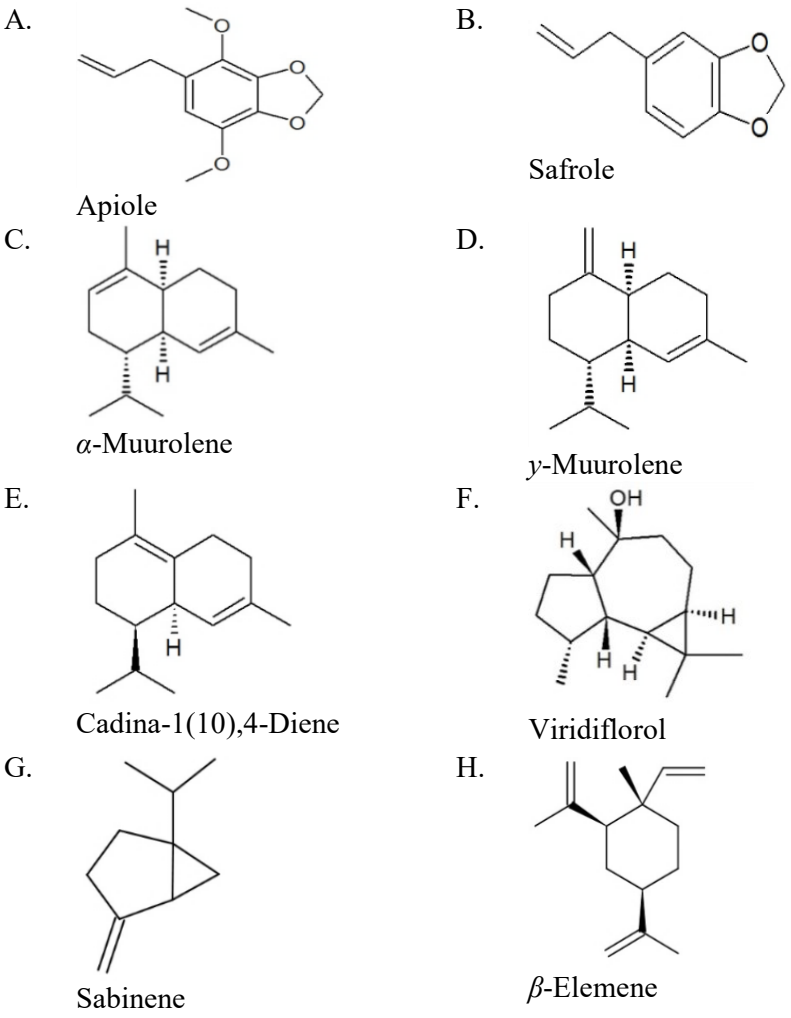


Figure 1. Chemical structures of the major compounds identified in the essential oils of *Piper aduncum*, *P. anonifolium*, *P. crassinervium* and *P. hispidinervum*.

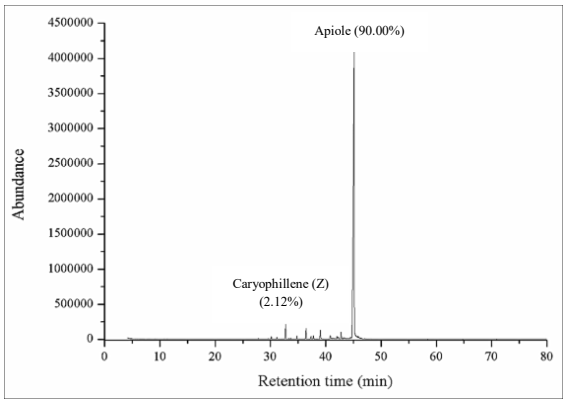
Two constituents were identified in the EO of *P. hispidinervum*, with the phenylpropanoid Safrole (98.80%) as the main compound, and Bicyclogermacrene (1.17%) was also present (Table 2, Figure 2D). This constituent was also found in the EO of leaves of this species collected in Lavras, MG, Brazil (83.00%) (LIMA et al., 2014). In plants cultivated in the state of Acre, Brazil, the EO showed a safrole concentration above 90% (FAZOLIN et al., 2007).

Caryophyllene (Z) occurred in three species, with the highest proportion in *P. anonifolium* (5.60%), followed by *P. crassinervium* (3.30%) and *P. aduncum* (2.10%). Bicyclogermacrene was found in *P. hispidinervum* (1.17%) and *P. aduncum* (0.53%). The constituents *α*-Copaene (0.36 and 0.66%), *β*-Elemene (0.28 and 7.07%), Germacrene (D) (1.62 and 2.61%) and Spathulenol (0.35 and 0.49), respectively, were common in the species *P. aduncum* and *P. crassinervium*. For *P. anonifolium* and *P. crassinervium*, the common compounds are *α*-Cubebene (1.61 and 1.33%), *β*-Humulene (0.46 and 0.88%), *γ*-Muurolene (16.6 and 1.01%), *α*-Muurolene (23.11 and 0.74%), Torreyol (4.10 and 0.92%) and Epi-*α*-Cadinol (5.16 and 2.59%), respectively.

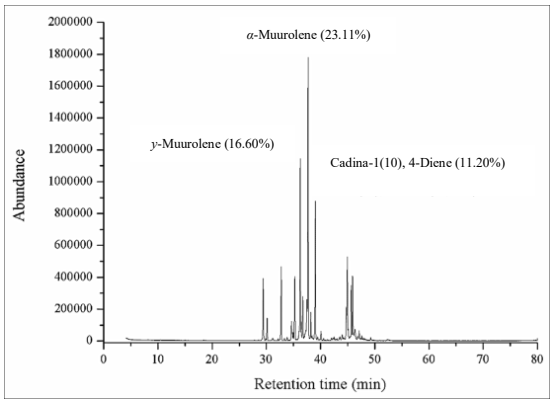
Among the major compounds found in the EOs, Safrole has insecticidal and antimicrobial properties and is used in the fragrance industry (SOARES et al., 2011). Apiol has insecticidal activity and, on a clinical basis, can act as antifungal, acaricide, antioxidant and anticancer. However, its prolonged ingestion, in addition to miscarriage, can cause chronic problems with the liver and kidney or anemia (TABASSUM; AKRAM; MUSHTAQ, 2021).

α-Muurolene and *γ*-Muurolene have pharmacological properties, as they exhibit antimicrobial activities (CHAIBUB et al., 2013). Viridiflorol promotes insecticidal action (ABOA; SERI-KOUASSI; KOUA, 2010), as well as fungitoxic, anti-inflammatory, anticancer, and antioxidant activity (AKIEL, et al., 2022). Sabinene, in turn, exhibits antifungal, antimicrobial and anticancer properties (NAGEEB; AZEIZ, 2018). The compound Cadina-1(10),4-Diene has an insecticidal effect and antimicrobial properties (PÉREZ-LÓPEZ et al., 2011). The occurrence of these compounds demonstrates that the EOs of these species are a promising source of potential new biopesticide ingredients, as well as for pharmacology, perfumery and medicine.

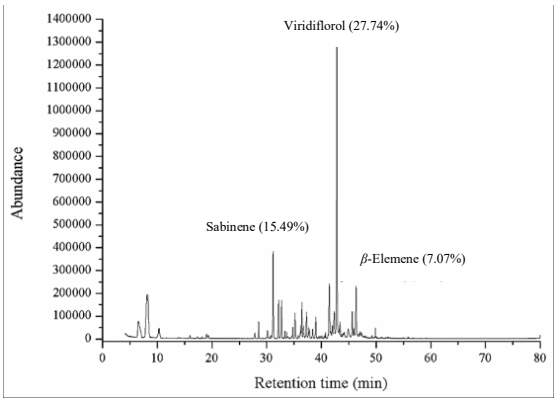
A. *Piper aduncum* L.



B. *Piper anonifolium* Kunth



C. *Piper crassinervium* Kunth



D. *Piper hispidinervum* C. DC

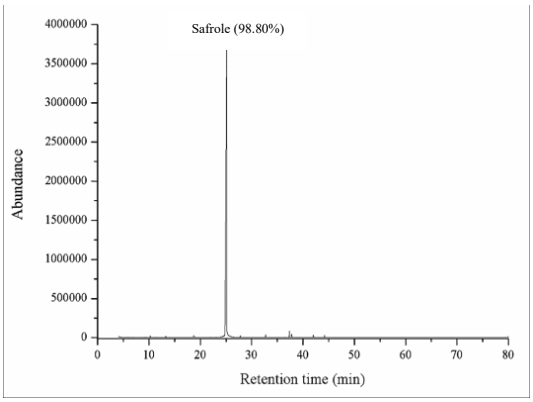


Figure 2. Chromatogram of the essential oils of *Piper aduncum* (A), *P. anonifolium* (B), *P. crassinervium* (C) and *P. hispidinervum* (D).

Mortality bioassays

The mortality of *A. monuste orseis* varied significantly between EOs ($F_{3;48}$=85.66; P≤0.0001), between the concentrations of EOs ($F_{1;48}$=182.48; P≤0.0001) and there was an interaction between these two factors ($F_{3;48}$=85.66; P≤0.0001). At a concentration of 157.25 nL cm^{-2}, all EOs were effective, causing 100% mortality. At the concentration of 2.60 nL cm^{-2}, the EOs of *P. anonifolium* and *P. aduncum* caused mortality of 100% and 97%, respectively, being significantly higher (P≤0.01) than the mortality rates caused by the EOs of *P. hispidinervum* (71%) and *P. crassinervium* (11%) (Figure 3A).

For *A. sexdens*, mortality varied significantly between EOs ($F_{3;72}$=25.86; P≤0.0001), between the concentrations of EOs ($F_{1;72}$=1102.29; P≤0.0001) and there was an interaction between these two factors ($F_{3;72}$=18.30; P≤0.0001). At the concentration of 157.25 nL cm^{-2}, the EOs of the species *P. hispidinervum* and *P. aduncum* showed significantly higher efficacy (P≤0.01), with 100% mortality, followed by the EOs of *P. anonifolium* and *P. crassinervium*, with 63% mortality. At the concentration of 2.6 nL cm^{-2}, the EOs had a low toxic effect, and mortality ranged from 2 to 12% (Figure 3B). During the bioassays, it was found that the EO of *P. aduncum* at a concentration of 2.60 nL cm^{-2} caused neurotoxic effects on leaf-cutting ants, which showed motor imbalance, such as tremors throughout the body, inability to bite and difficulty in locomotion.

For *Z. subfasciatus*, mortality varied significantly between EOs ($F_{3;24}$=54.19; P≤0.0001), between the concentrations of EOs ($F_{1;24}$=1728.82; P≤0.0001) and there was an interaction between these two factors ($F_{3;24}$=49.70; P≤0.0001). At the concentration of 157.25 nL cm^{-2}, the EO of *P. hispidinervum* caused 100% mortality, which is significantly higher (P≤0.01) than that caused by the EOs of *P. aduncum* (81%), *P. anonifolium* (76%) and *P. crassinervium* (65%). At the concentration of 2.60 nL cm^{-2}, the EOs of *P. aduncum* and *P. anonifolium* caused mortality of 41% and 26%, respectively, which were significantly higher (P≤0.01) than the rates caused by the EOs of *P. hispidinervum* (15%) and *P. crassinervium* (11%) (Figure 4A).

The mortality of *C. ferrugineus* varied significantly between EOs ($F_{3;24}$=17.00; P≤0.0001), between the concentrations of EOs ($F_{1;24}$=17.00; P≤0.0004) and there was an interaction between these two factors ($F_{3;24}$=17.00; P≤0.0001). All EOs at a concentration of 157.25 nL cm^{-2}

caused 100% mortality. At a concentration of 2.60 nL cm^{-2}, the EOs of *P. hispidinervum*, *P. aduncum* and *P. anonifolium* resulted in a mortality rate of 100%, which was significantly higher ($P \leq 0.01$) than the rate caused by the EO of *P. crassinervium* (66%) (Figure 4B).

Mortality of *S. zeamais* varied significantly between EOs ($F_{3;24}$=249.12; $P \leq 0.0001$), between the concentrations of EOs ($F_{1;24}$=492.59; $P \leq 0.0001$) and there was an interaction between these two factors ($F_{3;24}$=249.14; $P \leq 0.0001$). At the concentration of 157.25 nL cm^{-2}, the EO of *P. hispidinervum* caused 100% mortality, which was significantly higher ($P \leq 0.01$) than the mortality caused by the EO of *P. aduncum* (33%). The EOs of *P. anonifolium* and *P. crassinervium* showed no toxicity, causing 0% mortality. At a concentration of 2.60 nL cm^{-2}, the EOs were not lethal to *S. zeamais* or caused low mortality (0%) (Figure 4C).

In general, the EO of *P. hispidinervum* caused the mortality of 100% of the insects of the five species evaluated, using a concentration of 157.25 nL cm^{-2}. The insecticidal activity of *P. hispidinervum* was also observed for the species *Thyrinteina arnobia* (Stoll) (Lepidoptera: Geometridae) (SOARES et al., 2011), *Z. subfasciatus* (BRITO et al., 2012), *S. zeamais* and *Tenebrio molitor* L. (Coleoptera: Tenebrionidae) (FAZOLIN et al., 2007).

P. aduncum oil caused mortality of 100% in *A. monuste orseis*, *A. sexdens* and *C. ferrugineus*, 81% in *Z. subfasciatus* and 33% in *S. zeamais*, at a concentration of 157.25 nL cm^{-2}. The insecticidal activity of *P. aduncum* was also observed in *Solenopsis saevissima* (Hymenoptera: Formicidae) (SOUTO et al., 2012) and *Z. subfasciatus* (BRITO et al., 2012). On the other hand, the EO of *P. anonifolium* caused mortality of 100% in *A. monuste orseis* and *C. ferrugineus*, 63% in *A. sexdens* and 76% in *Z. subfasciatus*. According to Ruiz-Vásquez et al. (2022), the EO of *P. anonifolium* showed strong antifungal activity.

It was found that the EO of *P. crassinervium* caused mortality of 100% in *A. monuste orseis* and *C. ferrugineus*, 63% in *A. sexdens*, 65% in *Z. subfasciatus* and was non-toxic for *S. zeamais*. The insecticidal activity of *P. crassinervium* has been observed against *Anticarsia gemmatalis* (Lepidoptera: Noctuidae) and *S. zeamais* (KRINSKI; FOERSTER; DESCHAMPS, 2018), corroborating the results obtained in this investigation.

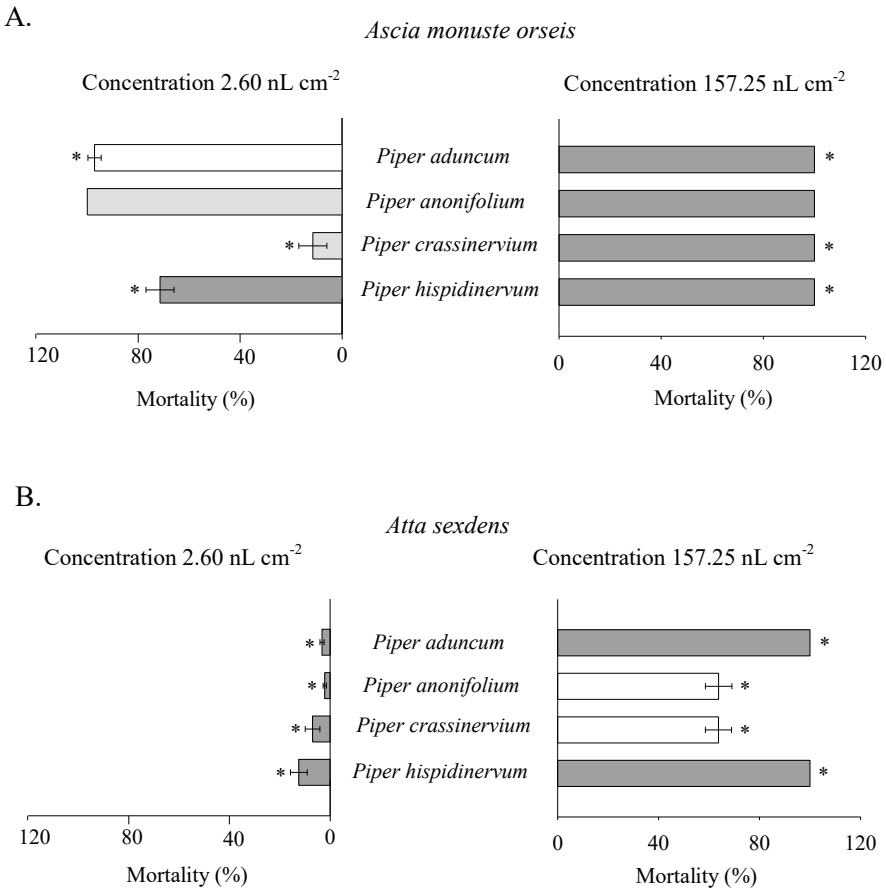


Figure 3. Mortality (%) of *A. monuste orseis* (A) and *A. sexdens* (B) at concentrations of 2.60 and 157.25 nL cm^{-2}. Means grouped with bars of equal colors did not differ significantly between plant species and asterisks indicate significant difference between concentrations by Tukey test (P<0.05).

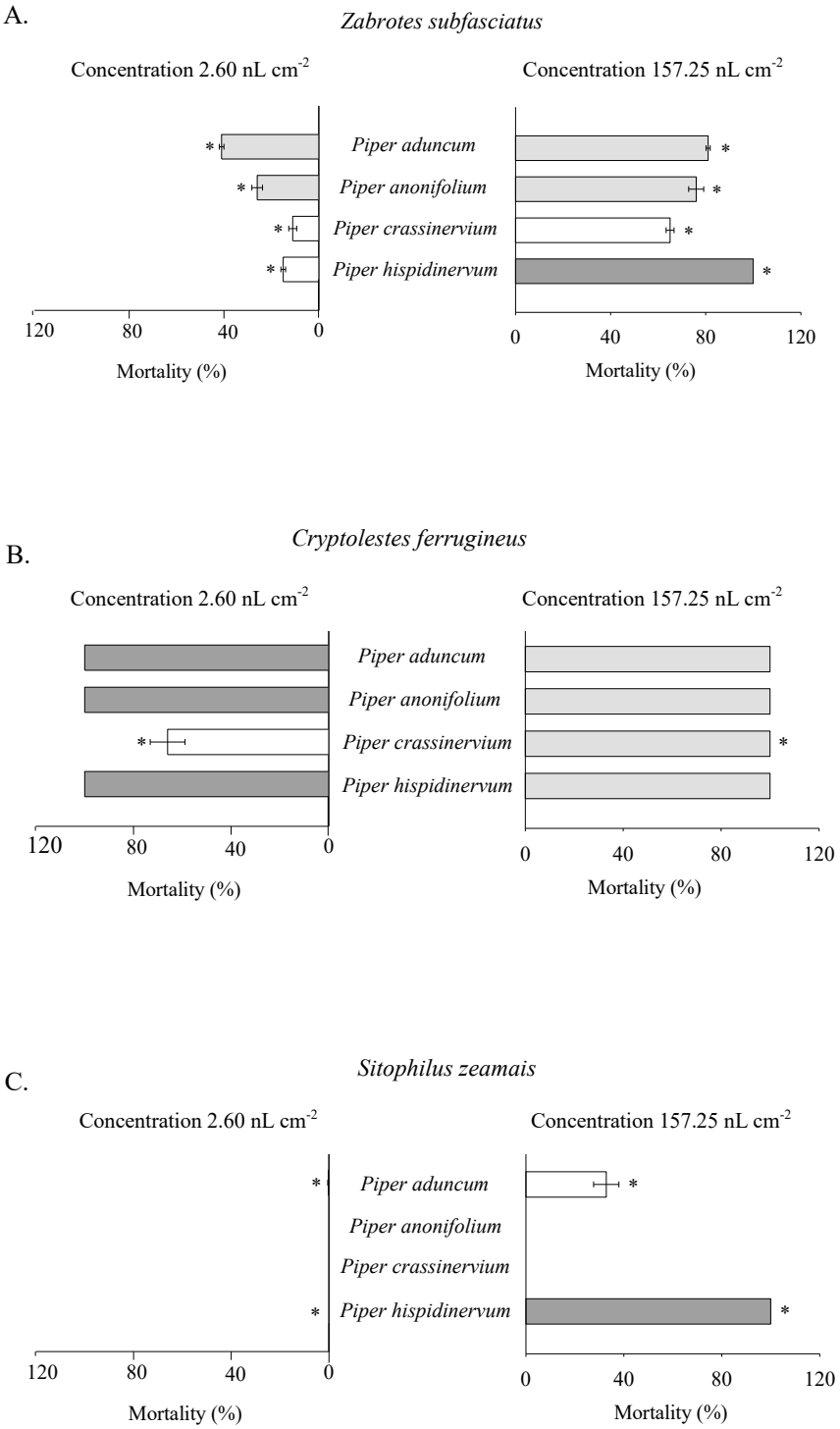


Figure 4. Mortality (%) of *Z. subfasciatus* (A), *C. ferrugineus* (B) and *S. zeamais* (C) at concentrations of 2.60 and 157.25 nL cm^{-2}. Means grouped with bars of equal colors do not differ significantly between plant species and asterisks indicate significant difference between concentrations by Tukey test (P<0.05).

The Piperaceae species investigated have potential for obtaining new molecules with bioinsecticidal activity. These results are of great relevance to the toxicology of new insecticides and increase the primary information on the EOs of Piperaceae species in the control of *A. monuste orseis* great southern white caterpillars, *A. sexdens* leaf-cutting ants, *Z. subfasciatus* bean weevil, *C. ferrugineus* beetle and *S. zeamais* maize weevil. It is worth pointing out that only *P. hispidinervum* and *P. aduncum* were effective for *S. zeamais* mortality. Several studies also address the use of plants of the

genus *Piper* as potential synergists (DUROFIL et al., 2021; FAZOLIN et al., 2016; OLIVEIRA et al., 2023). An alternative for application in the control of these pests is through spraying, with potential for developing evident formulations in the pesticide industry.

CONCLUSIONS

The yield of EOs was higher in the species *P. aduncum* and *P. hispidinervum*, with drying in an oven at 45 °C.

The major constituents present in the EOs of *P. hispidinervum* and *P. aduncum* were safrole (98.80%) and apiol (90.00%). For *P. anonifolium*, the major constituents were *α*-Muurolene (23.11%), *γ*-Muurolene (16.60%), Cadina-1(10) and 4-Diene (11.2%).

For *P. crassinervium*, the major constituents were Viridiflorol (27.7%) and Sabinene (15.5%).

In general, the EO of the *Piperaceae* species showed a toxic effect on pest insects and are potential sources for implementation in integrated pest management.

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