ACTA
AMAZONICA

http://dx.doi.org/10.1590/1809-4392202102292

ORIGINAL ARTICLE

Insecticidal activity of *Piper aduncum* oil: variation in dillapiole content and chemical and toxicological stability during storage

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ABSTRACT

The insecticidal effect of the essential oil of *Piper aduncum* (EOPA), and of its constituent dillapiole [1-allyl-2,3-dimethoxy-4,5-(methylenedioxy) benzene] in particular, is well documented in the literature and can be associated with its interference with the enzymatic detoxification in arthropods. However, no data exist on the range of dillapiole content associated with insecticidal activity, which is necessary to establish reliable dose-activity parameters for a formulated product. The oil composition can also change during storage after distillation, mainly due to environmental factors such as light incidence, atmospheric oxygen and temperature, which can be deleterious to oil quality. In this study, EOPA subjected to different storage conditions over four years and its rectified fractions were submitted to bioassays to evaluate their insecticidal effect by topical contact and residual contact against *Spodoptera frugiperda*. Our objectives were to determine the relationship between dillapiole content and the insecticidal activity of EOPA, and to evaluate its chemical and toxicological properties over time under different conditions. Our results showed that EOPA was stable with respect to the dillapiole content and the toxicological effect against *S. frugiperda* under different storage conditions for four years. The overall chemical composition of the EOPA did not vary significantly among storage conditions. EOPA with dillapiole content ranging between 68% and 100% showed greater insecticidal toxicity by residual and topical contact against *S. frugiperda* larvae.

KEYWORDS: chemical and toxicological stability, arylpropanoids, botanical insecticides, terpenoid storage, 1-allyl-2,3-dimethoxy-4,5-(methylenedioxy) benzene

Atividade inseticida do óleo de *Piper aduncum*: variação no conteúdo de dilapiol e estabilidade química e toxicológica durante o armazenamento

RESUMO

O efeito inseticida do óleo essencial de *Piper aduncum* (OEPA) e, particularmente, de seu constituinte dilapiol [1-alil-2,3-dimetoxi-4,5-(metilenodioxi) benzeno], está bem documentado na literatura e pode estar associado à sua interferência na desintoxicação enzimática em artrópodes. No entanto, não existem dados sobre a amplitude de teores de dilapiol associados à atividade inseticida, o que é necessário para estabelecer parâmetros de dose-atividade confiáveis para um produto formulado. A composição do óleo também pode sofrer alterações durante seu armazenamento após a destilação, principalmente devido a fatores ambientais como incidência de luz, oxigênio atmosférico e temperatura, que podem ser deletérios à qualidade do óleo. Neste estudo, durante quatro anos, OEPA submetido a diferentes condições de armazenamento e suas frações retificadas foram submetidos a bioensaios para avaliar seu efeito inseticida por contato tópico e contato residual contra *Spodoptera frugiperda*. Nossos objetivos foram determinar a relação entre o teor de dilapiol e a atividade inseticida do OEPA, e avaliar suas propriedades químicas e toxicológicas ao longo do tempo sob diferentes condições. Nossos resultados mostraram que o OEPA foi estável em relação ao teor de dilapiol e o efeito toxicológico contra *S. frugiperda* sob diferentes condições de armazenamento durante quatro anos. A composição química do OEPA não variou significativamente entre as condições de armazenamento. OEPA com teor de dilapiol entre 68% e 100% apresentou maior toxicidade inseticida por contato residual e tópico contra larvas de *S. frugiperda*.

PALAVRAS CHAVES: estabilidade química e toxicologica, arilpropanoides, inseticidas botânicos, armazenamento de terpenóides, 1-alil-2,3-dimetoxi-4,5-(metilenodioxi) benzeno

CITE AS: Fazolin, M.; Monteiro, A.F.M.; Bizzo, H.R.; Gama, P.E.; Viana, L.O.; Lima, M.É.C. 2021. Insecticidal activity of *Piper aduncum* oil: variation in dillapiole content and chemical and toxicological stability during storage. *Acta Amazonica* 52: 179-188.

INTRODUCTION

The essential oil of *Piper aduncum* L. (EOPA) has an excellent yield (2.5 to 3.5%) in comparison with other essential oils, and is rich in dillapiole (31.5 to 91.1%), a allylphenol with a high oxygenation pattern (1-allyl-2,3-dimethoxy-4,5- (methylenedioxy) benzene) (Maia *et al.* 1998). The insecticidal effect of EOPA, and of dillapiole in particular, is well documented in the literature and can be associated to its interference with arthropod enzymatic detoxification, as reviewed by Durofil *et al.* (2021), who reported 23 arthropods of agricultural and livestock importance controlled by *P. aduncum* compounds.

The composition and thus the biological activity of essential oils vary batch-to-batch (Morais 2009). Associative effects and different action forms are observed between major and minor component activities, as well as synergistic effects (Bakkali *et al.* 2008). These effects must be evaluated on a case-by-case basis for any particular oil composition. Arylpropanoids, like dillapiole, can act by inhibiting enzymatic pathways, while terpenic compounds can be neurotoxic or, due to their lipophilicity, facilitate the action of other oil constituents to cross insects cuticle (Afshar *et al.* 2017).

EOPA activity is associated with the presence of dillapiole (Estrela *et al.* 2006). However, there are no data on the relation between content and activity level of dillapiole. This knowledge is necessary to establish reliable dose-activity parameters for formulated products. The quantitative and qualitative composition of EOPA varies with genetic and geographic intraspecific variability, e.g., in the western Brazilian Amazon, EOPA can contain from 18 to 56 components (Andrade *et al.* 2009).

The essential oil composition can also change during storage, after distillation, mainly due to environmental factors such as light incidence, atmospheric oxygen and temperature (Turek and Stintzing 2012). The effect of these factors can be deleterious to oil quality, particularly due to the formation of hydroperoxides (Choe and Min 2006). Molecular rearrangement and thermal degradation were also observed in the absence of oxygen (Geier 2006).

Reliable and wide-scope data on essential oil storage are rare, and the accurate definition of shelf-life for most essential oils has not yet been established (Blitzke 2009; Turek and Stintzing 2012). When available, these data were obtained from oils rich in terpenic compounds, which are particularly volatile and reactive (Turek and Stintzing 2012). No data were found for arylpropanoid-rich oils, such as EOPA. Therefore, the objectives of this study were to establish the relation between the dillapiole content and the insecticidal activity of EOPA, and its chemical and toxicological stability under different storage conditions.

MATERIAL AND METHODS

Distillation and rectification of EOPA

Piper aduncum plants were harvested in April 2016 at a 0.5-ha production area at Embrapa Acre (10º15'57"S, 67º42'17"W), state of Acre, northern Brazil. Plants were cut 0.4 m above the ground, the leaves were removed and dried in an oven at 20°C to 35°C until reaching 30% of moisture. In accordance with Brazilian legislation, access to native germplasm was authorized by the Ministry of the Environment (SisGen licenses: processes # 02001.006140/2011-78; 02000.000644/2013-56; 02000.000460/2013-96; 02000.002056/2014-38).

The essential oil was obtained by steam distillation of the leaves for approximately four hours in a 200-L still (ERCITEC, Bauru -SP, Brazil) in batches of 150 kg. The oil obtained was dried over anhydrous magnesium sulphate and had a dillapiole content of 78%. The distilled oil was rectified by fractional distillation in a 30-L still (ERCITEC, Bauru-SP, Brazil). Thirteen fractions, distilling from 270 °C to 350 ºC under atmospheric pressure (760 mmHg), were collected at 1-hour intervals, with increasing concentrations of dillapiole of 12.3, 22.1, 32.2, 42.1, 52.3, 62.4, 67.3, 72.1, 77.3, 82.2, 87.2, 94.1 and 99.8%. The fractions were stored in a domestic refrigerator at 6 °C until the beginning of the experiment in May 2016.

Analysis of EOPA and its fractions

The EOPA and its rectified fractions were analyzed by gas chromatography in an Agilent 7890A gas chromatograph (GC) fitted with a 7693B automatic sampler and a flame ionization detector (FID). Samples were diluted to 1% with dichloromethane and 1 µL was injected at 250 ºC in split mode (1:50). The FID was operated at 280 ºC. Separation of the components was obtained with a HP-5MS fused silica capillary column (5%-phenyl-95%-methyl-silicone, 30 m x 0.25 mm x 0.25 µm). Hydrogen was used as carrier gas (1.5 ml min^{-1}). Oven temperature was programed from 60 to 240 ºC at 3 ºC min^{-1}. Quantification was based on the area (area %) from the signal of the FID normalized with an internal standard. All analyses were made in triplicate. All compounds > 0.1 area % were quantified in the oil composition, while compounds < 0.1 area % were considered as trace elements.

Analyses by mass spectrometry (GC-MS) were performed on an Agilent 5975C mass selective detector coupled to an Agilent 7890A gas chromatograph with the same column, temperatures and injection conditions as above. Helium was used as carrier gas at 1 mL min^{-1}. The mass detector was operated in electron ionization mode (70 eV), at 3.15 scans sec^{-1}, with mass range from 40 to 450 u. The transfer line was kept at 240 ºC, the ion source at 230 ºC and the analyzer at 150 ºC.

The identification of the oil components was performed by comparison of their mass spectra with those from the Wiley Registry of Mass Spectral Data (McLafferty 1994) or NIST databases (NIST 2011), as well as their linear retention indices (LRI), calculated according to Van Den Dool and Kratz (1963), after the injection of a homologous series of hydrocarbons (n-C_7-C_{26}) in the same conditions as above, and compared to literature data (Joulain and König 1998; Adams 2007).

Degradation of EOPA

The experimental methodology to evaluate the degradation of EOAP was adapted from Turek and Stintzing (2012).

We tested four storage conditions: (a) exposure to ultraviolet radiation in a UV chamber; (b) exposure to direct sunlight; (c) indoors at room temperature at Embrapa Acre; and (d) uncovered in a domestic refrigerator. Eight vials containing 5 ml of EOPA with 78% dillapiole were used for each treatment (four colorless vials and four amber glass vials). A control consisted of four amber glass vials covered with aluminum foil stored in a refrigerator, to eliminate any interference of the refrigerator light when opening the refrigerator door. In total, 36 vials were used. The samples were coded according to Table 1.

The exposure period for all storage conditions was four years, from April 2016 to April 2020. Each year (at days 0, 360, 720, 1080 and 1440) one vial of each color was removed for chemical characterization and toxicological assays, using a sub-sample of 2 ml from each vial for each analysis.

A UV chamber was adapted from a laminar flux chamber fitted with an Actinica Philips model TLD15W/03 ultraviolet lamp (450 mm, 15 W, range: 380-480 nm), for simulation of UV-A and UV-B radiation. The lamp was kept lit permanently. An air conditioning system kept the temperature at an average 25.9 ºC (minimum 22.8 ºC, maximum 29 ºC). The indoors samples were kept in a 20-m^2 room with no temperature control. Average temperature in the room was 26.7 ºC (minimum 19.7 ºC, maximum 30.3 ºC). Direct exposure to sunlight was approximately 12 h day^{-1} (6:00 am to 6:00 pm), with average temperature of 29.8 ºC (minimum 22.6 ºC, maximum 50.7 ºC). Temperature data were obtained using a data logger (Escort RH iLog, range -40 to 70º C) for each experimental condition.

Table 1. Sample codes for the experimental treatments of four-year degradation of *Piper aduncum* essential oil.

Treatment	Code
UV chamber, amber flask	UVCA
UV chamber, colorless flask	UVCC
Indoors, amber flask	INAF
Indoors, colorless flask	INCF
Refrigerator, amber flask	REFA
Refrigerator, colorless flask	REFC
Direct sunlight exposition, amber flask	SUNA
Direct sunlight exposition, colorless flask	SUNC
Control (refrigerator, amber flask covered with aluminium foil)	CONT

In vitro toxicological effect of EOPA

After fractional distilation, the 13 EOAP fractions with different dillapiole contents were submitted to bioassays to evaluate the insecticidal effect by topical contact and by residual contact in May 2016. The same assays were also performed each year with the storage-conditions samples. Larvae of *Spodoptera frugiperda* (JE Smith, 1797), which is a common agricultural pest in tropical and subtropical regions, were used as a target insect.

The toxicological evaluations were carried out at the Entomology Laboratory of Embrapa Acre and followed the methodology of Estrela *et al*. (2006).Third instar larvae were used in all bioassays (breeding authorization by SISBIO license # 13464-2).

The experimental parameters for the bioassays were determined in preliminary tests following the methodology of Robertson *et al*. (2016) using a completely randomized design with four repetitions per treatment. Each replicate consisted of 10 insect larvae in a Petri dish. For all 13 EOPA fractions and the yearly samples of EOAP from the storage-condition treatments, we used doses of 0.2 mL EOPA per larva for residual contact and 1 µL for topical contact, followed by 24 h without feeding. After determining the overall response range from concentrations that caused nearly zero to nearly 100% mortality of larvae, narrower response ranges were determined, following the methodology described by Finney (1971). Seven concentrations were selected through this methodology for the final residual and topical contact bioassays.

In the final residual and topical contact bioassays, the selected concentrations and doses were used for all 13 fractions and annual sub-samples of storage-condition treatmentswith four repetitions per treatment. Each replicate consisted of 10 insect larvae in a Petri dish. For the assay on topical contact, 1 µL of the test sample was applied on the dorsal side of the larva´s pronotum with the aid of a graduated micro syringe (Al-Sarar *et al*. 2006). For the assay on residual contact, a filter paper of 5 cm diameter impregnated with 0.2 mL of the test sample was dried in a fume hood for 5 min until the solvent had completely evaporated (Estrela *et al*. 2006) and then placed in a Petri dish that received a larva. In both assays, acetone solvent was used as a negative control, and the treated larvae were individualized and left without food in Petri dishes (5.0 cm × 1.5 cm) and placed and maintained in a thermoelectric refrigerated incubator at 25° C ± 2 °C, 70 ± 5% relative humidity, and 12 h photophase. After 24 hours larval mortality was assessed.

Statistical analysis

The observed mortality in the toxicological assays was corrected for natural mortality using Abbott´s correction (Abbott 1925).

For the determination of LC_{50} and LD_{50}, respectively the concentration (residual contact) and dose (topical contact) more likely to result in a 50% mortality of the larvae, concentration-mortality curves and confidence intervals (95% CI) were determined by Probit analysis using the SAS program (SAS Institute 2001). To test the goodness-of-fit, Pearson's chi-square test (χ^2) was used with a significance level of 5%.

The overlap among the confidence intervals (95% CI) for LC_{50} and LD_{50} was used to define the significance of the differences among the treatments according to Van Frankenhuyzen (2019). The overlap of the confidence intervals was determined visually and by comparing the maximum and minimum values of each IC within each experiment (13 fractions of EOPA with different dillapiole concentrations and the annual subsamples of storage-condition treatments).

The mortality-response data of the *S. frugiperda* larvae as a function of dillapiole content of the 13 EOPA fractions were fitted to a quadratic function and the range of dillapiole contents that did not differ significantly in larvae mortality was defined from the inflection points of the curve. The analysis was done with SYMBOLAB (2020) (EqsQuest Ltd).

RESULTS

There was little variation in the oil composition of the control sample, even after four years of storage (Table 2). The main variation was observed for monoterpenes (α-pinene, β-pinene, α-phellandrene and β-ocimene), which had lower concentrations after 1440 days than at the beginning of the storage period. This can be explained by the slow evaporation of these very volatile components.

During storage, as the lighter compounds evaporated, the relative proportional areas changed, and the concentrations of several sesquiterpenes, oxygenated sesquiterpenes and even arylpropanoids, originally present in the oil as trace compounds (< 0.1%), rose above the 0.1% threshold and were included in the EOPA composition. The initial sesquiterpene quantification (peaks 5-9) did not change expressively during storage, but there was a small increase in the percentage of oxygenated sesquiterpenoids, such as caryophyllene oxide (1.0 to 1.4%) and humulene epoxide II (trace to 0.1%), which is compatible with the aging of the oil. The small reduction in the relative amount of dillapiole was likely due to the increase in quantifiable compounds over time. Therefore, when kept under refrigeration (thus in the dark), in a hermetically closed bottle, regardless of the color of the bottle, the EOPA was chemically stable for at least four years, similarly to the control.

Monoterpenes were lost under the more adverse storage conditions, and after four years they were found only in the samples kept under refrigeration, including the control (Table 3). The sesquiterpene content decreased under non-refrigerated storage, mainly in the colorless flasks exposed to UV and sunlight, particularly for (*E*)-caryophyllene, α-humulene, bicyclogermacrene and germacrene D. Among the oxygenated sesquiterpenoids, the content of alcohols (nerolidol, spathulenol, viridiflorol) varied little among treatments (Table 3).

The epoxides such as caryophyllene oxide and humulene epoxide generally increased throughout time, with a higher increase in the samples exposed to highest radiation (UVCC and SUNC). A small decrease observed in pentadecane content can be associated to evaporation in the samples kept outside refrigeration (UVCA, UVCC, WARA, WARC, SUNA and SUNC). No relevant variation was observed in arylpropanoid content (Table 3, Supplementary Material, Tables S1,S2, S3).

The confidence intervals of LD_{50} and LC_{50} values largely overlapped among treatments and over time (Figures 1 and 2), indicating that the toxicity of 4-year stored EOPA against *S. frugiperda* larvae did not differ significantly from the fresh EOPA, which reflects the little variation observed in the EOPA composition over the storage time.

The fitted curve of LC_{50} values for dillapiole fractions had a high coefficient of determination ($R^2 = 0.9414$) (Figure 3). The inflection point corresponded to a dillapiole content of 77.8% (LC_{50} = 0.0055 µL cm^{-2}). There was no significant difference in larval mortality in the range of 67 to 82% dillapiole, corresponding to an overlap of the confidence intervals of LC_{50} of 0.5586 - 0.0071 µL cm^{-2} and 0.0065 - 0.0074 µL cm^{-2}, with $p > 0.05$ for Pearson's chi-square test (determined by Probit analysis). This means that the concentration of dilapiol in the range of 67 to 82% has a linear toxicological response to residual contact against *S. frugiperda* larvae. The model indicated that concentrations of dillapiole between 68% and 88% promote the highest residual contact toxicity to this insect.

The fitted curve of LD_{50} values for dillapiole fractions also had a high coefficient of determination ($R^2 = 0.9609$) (Figure 4). The inflection point corresponded to a dillapiole content of 100.0% (LD_{50} = 0.0024 µL mg^{-1} of insect weight). There was no significant difference in larval mortality in the range of 67 to 87% dillapiole, corresponding to an overlap of the confidence intervals of LD_{50} of 0.0032 - 0.0037µL mg^{-1} of insect weight and 0.0029 - 0.0035 µL mg^{-1} of insect weight, with $p > 0.05$ for Pearson's chi-square test (determined by Probit analysis). The concentration of dilapiol in the range of 67 to 87% also had a linear toxicological response to topical contact against *S. frugiperda* larvae. The model indicated that

Table 2. Composition (area %) of a sample of essential oil of *Piper aduncum* from Acre state, Brazil during four years of storage in an amber flask wrapped in aluminium foil in a domestic refrigerator (control sample).

Peak	LRI_{calc}	LRI_{lit}	Identified compounds	Time (days)*				
				0	360	720	1080	1440
1	932	932	α-pinene	0.7	0.7	0.6	0.5	0.1
2	976	974	β-pinene	1.2	1.2	1.2	1.1	0.5
3	1005	1002	α-phellandrene	0.4	0.4	0.3	0.3	0.2
4	1022	1020	*p*-cymene	tr	0.1	tr	tr	0.1
5	1026	1024	limonene	tr	0.3	0.2	0.2	0.2
6	1035	1032	(*Z*)-β-ocimene	tr	tr	tr	tr	0.1
7	1046	1044	(*E*)-β-ocimene	0.5	tr	tr	tr	0.3
8	1345	1345	α-cubebene	tr	tr	tr	tr	tr
9	1362	1369	cyclosativene	tr	tr	0.2	0.2	0.2
10	1374	1374	α-copaene	1.0	1.1	1.1	1.1	1.1
11	1389	1389	β-elemene	tr	tr	0.1	0.1	0.3
12	1404	1409	α-gurjunene	tr	tr	tr	tr	0.1
13	1415	1417	(*E*)-caryophyllene	8.0	9.0	9.1	9.1	8.9
14	1423	1430	β-copaene	tr	tr	tr	tr	0.1
15	1433	1439	aromadendrene	tr	tr	tr	tr	0.3
16	1448	1449	α-humulene	1.0	1.1	1.2	1.1	1.1
17	1455	1457	β-santalene	tr	0.5	0.5	0.5	0.5
18	1472	1477	γ-muurolene	tr	tr	tr	tr	0.1
19	1479	1480	germacrene D	0.9	1.1	1.1	1.0	1.0
20	1486	1489	β-selinene	tr	tr	tr	tr	0.2
21	1490	1493	(*E*)-muurola-4(14),5-diene	tr	tr	tr	tr	0.1
22	1494	1494	bicyclogermacrene	1.0	1.1	1.1	1.1	1.0
23	1497	1500	pentadecane	1.7	1.7	1.7	1.7	1.8
24	1506	1511	δ-amorphene	0.5	0.4	0.4	0.4	0.3
25	1515	1514	cubebol	0.8	0.8	0.8	0.5	0.6
26	1519	1517	myristicin	2.4	2.6	2.1	2.0	2.5
27	1528	1533	(*E*)-cadina-1,4-diene	tr	tr	tr	tr	0.2
28	1538	1544	α-calacorene	tr	tr	tr	0.3	0.3
29	1556	1555	elemicin	tr	tr	tr	tr	0.4
30	1561	1561	(*E*)-nerolidol	tr	0.4	0.4	0.4	0.6
31	1573	1577	spathulenol	tr	0.4	0.5	0.6	0.5
32	1578	1582	caryophyllene oxide	1.0	1.2	1.3	1.3	1.4
33	1586	1592	viridiflorol	0.8	0.9	0.9	0.9	0.9
34	1604	1608	humulene epoxide II	tr	tr	tr	tr	0.1
35	1619	1620	dillapiole	78.0	74.1	74.8	75.7	73.5
36	1677	1677	apiole	tr	tr	tr	tr	0.2
			Total	99.9	99.1	99.6	100.0	99.7
			Monoterpenes	2.8	2.7	2.3	2.1	1.5
			Oxygenated monoterpenoids	0.0	0.0	0.0	0.0	0.0
			Sesquiterpenes	13.2	14.3	15.6	15.4	16.3
			Oxygenated sesquiterpenoids	1.8	3.3	2.6	2.6	3.0
			Arylpropanoids	80.4	76.7	76.9	77.7	76.6
			Others	1.7	1.7	1.7	1.7	1.8

LRI_{calc} (linear retention indices calculated) calculated according to Van Den Dool and Kratz (1963); LRI_{lit} (linear retention indices from the literature) calculated according to Adams (2007) and Joulain and König (1998); * limit of quantitation = 0.1 % area; tr = trace (< 0.1 % área).

concentrations of dillapiole between 82% and 100% promote the highest topical contact toxicity to this insect.

DISCUSSION

Previous data suggest that dillapiole is sensitive to degradation when stored (Tisserand and Young 2013), however, our results showed that, even after four years under the most adverse conditions, such as exposure to UV radiation, sunlight and high temperatures (above 40°C), the dillapiole content of EOPA was preserved. Therefore, storage periods of up to four years can be considered safe for the dillapiole content of EOPA shelf-life. In addition, caryophyllene oxide and humulene epoxide content increased with time, especially in the samples exposed to radiation in colorless glass, in agreement with the role of epoxides as typical markers for old and oxidized oils (Turek and Stintzing 2013; Najafian 2016; Albino *et al.* 2017).

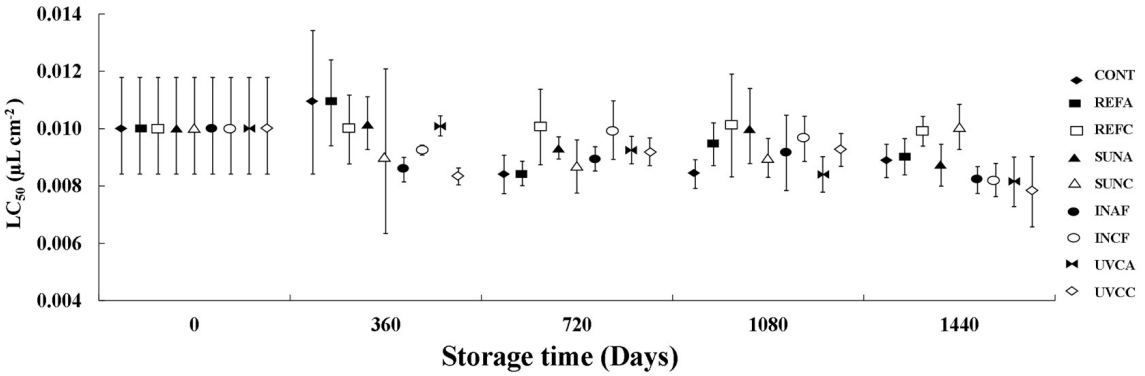

Figure 1. Lethal concentration of *Piper aduncum* essential oil subjected to different storage conditions by residual contact on *Spodoptera frugiperda* caterpillars. Symbols are the mean of four replicates and bars the 95% CI obtained by Probit analysis. CONT = sample stored in a domestic refrigerator, amber glass flask covered with aluminium foil; REFA = sample kept in a refrigerator at 6 ºC in an amber flask; REFC = sample kept in a refrigerator at 6 ºC in a colorless flask; SUNA = sample kept under sunlight exposition (approximately 12 hours per day) in an amber flask; SUNC = sample kept under sunlight exposition (approximately 12 hours per day) in a colorless flask; INAF= sample kept indoors at ambient temperature in an amber flask; INCF = sample kept indoors at ambient temperature in a colorless flask; UVCA = sample under UV light in an amber flask; UVCC = sample under UV light in a colorless flask.

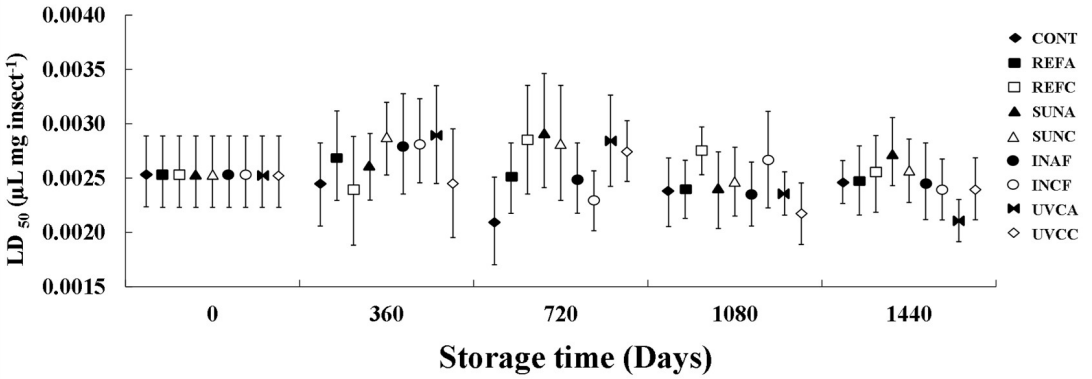

Figure 2. Lethal dose of *Piper aduncum* essential oil subjected to different storage conditions by topical contact on *Spodoptera frugiperda* caterpillars. Symbols are the mean of four replicates and bars the 95% CI obtained by Probit analysis. CONT = sample stored in a domestic refrigerator, amber glass flask covered with aluminium foil; REFA = sample kept in a refrigerator at 6 ºC in an amber flask; REFC = sample kept in a refrigerator at 6 ºC in a colorless flask; SUNA = sample kept under sunlight exposition (approximately 12 hours per day) in an amber flask; SUNC = sample kept under sunlight exposition (approximately 12 hours per day) in a colorless flask; INAF = sample kept indoors at ambient temperature in an amber flask; INCF = sample kept indoors at ambient temperature in a colorless flask; UVCA = sample under UV light in an amber flask; UVCC = sample under UV light in a colorless flask.

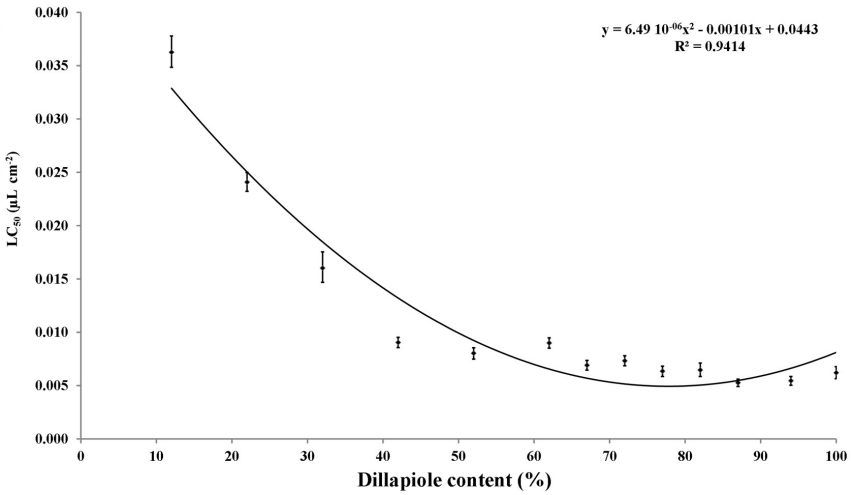

Figure 3. Toxicological effect by residual contact of fractions of *Piper aduncum* essential oil containing different dillapiole concentrations on *Spodoptera frugiperda* larvae. Points are the mean of four replicates and bars the 95% CI obtained by Probit analysis. R^2 = coefficient of determination.

Table 3. Constituents of the essential oil of *Piper aduncum* from Acre state (Brazil) after four years of storage under different conditions (in area %).

Peak	LRI_{calc}	LRI_{lit}	Identification	UVCA	UVCC	INAF	INCF	REFA	REFC	SUNA	SUNC	CONT
1	932	932	α-pinene	tr	tr	tr	tr	tr	tr	tr	tr	0.1
2	976	974	β-pinene	tr	0.1	tr	tr	tr	0.2	0.1	0.1	0.5
3	1005	1002	α-phellandrene	tr	0.2	tr	tr	0.1	0.1	tr	tr	0.2
4	1022	1020	*p*-cymene	tr	tr	tr	tr	tr	0.1	tr	tr	0.1
5	1026	1024	limonene	tr	tr	tr	tr	0.1	0.1	tr	tr	0.1
6	1035	1032	(*Z*)-β-ocimene	tr	tr	tr	tr	0.1	0.1	tr	tr	0.1
7	1046	1044	(*E*)-β-ocimene	tr	tr	tr	tr	0.2	0.2	tr	tr	0.3
8	1100	1100	linalool	tr	tr	tr	tr	0.1	tr	tr	tr	tr
9	1345	1345	α-cubebene	tr	0.1	tr	0.1	0.1	0.1	0.1	0.1	tr
10	1362	1369	cyclosativene	0.1	tr	0.1	0.1	0.2	0.2	0.1	0.1	0.2
11	1374	1374	α-copaene	0.4	0.5	0.7	0.7	1.1	1.1	0.6	0.8	1.1
12	1380	1387	β-bourbonene	tr	tr	tr	tr	tr	tr	tr	0.3	tr
13	1386	1387	β-cubebene	tr	tr	tr	tr	0.1	0.1	tr	tr	0.1
14	1389	1389	β-elemene	0.1	0.1	0.3	0.2	0.2	0.3	0.2	0.2	0.3
15	1404	1409	α-gurjunene	tr	tr	tr	tr	0.1	0.1	tr	tr	0.1
16	1409	1408	(*Z*)-caryophyllene	tr	tr	tr	tr	tr	tr	tr	0.1	tr
17	1415	1417	(*E*)-caryophyllene	4.9	4.1	6.6	6.6	8.7	8.9	5.1	2.2	8.9
18	1423	1430	β-copaene	0.1	tr	0.1	0.1	0.1	0.1	0.1	0.1	0.1
19	1433	1439	aromadendrene	0.2	tr	0.2	0.2	0.3	0.3	0.2	0.3	0.3
20	1448	1449	α-humulene	0.8	0.7	1.0	1.0	1.1	1.2	0.8	0.6	1.1
21	1455	1457	β-santalene	0.3	0.3	0.4	0.4	0.5	0.5	0.4	0.4	0.5
22	1470	1475	(*E*)-cadina-1(6),4-diene	tr	tr	0.1	tr	tr	tr	0.1	tr	tr
23	1472	1477	γ-muurolene	0.1	tr	tr	0.1	0.1	0.1	tr	0.1	0.1
24	1479	1480	germacrene D	0.6	tr	0.7	0.7	1.0	1.0	0.3	tr	1.0
25	1480	1482	ar-curcumene	0.2	0.3	0.1	0.2	0.1	tr	0.2	0.3	tr
26	1486	1489	β-selinene	tr	tr	0.1	tr	0.1	0.2	tr	tr	0.2
27	1490	1493	(*E*)-muurola-4(14),5-diene	0.1	tr	0.1	tr	0.1	0.1	tr	tr	0.1
28	1493	1494	*epi*-cubebol	0.7	0.2	0.2	0.7	0.4	-	0.1	0.3	-
29	1494	1494	bicyclogermacrene	tr	tr	0.5	tr	0.6	1.0	0.3	tr	1.0
30	1497	1500	pentadecane	1.2	1.2	1.4	1.5	1.8	1.8	1.1	1.4	1.8
31	1502	1511	δ-amorphene	0.2	0.1	0.1	0.2	0.3	0.3	0.1	tr	0.3
32	1513	1514	β-curcumene	tr	tr	tr	tr	0.2	0.2	tr	tr	0.2
33	1515	1514	cubebol	0.2	0.2	0.5	0.5	0.6	0.6	0.4	0.3	0.6
34	1519	1517	myristicin	2.5	2.6	2.6	2.6	2.5	2.5	2.5	2.4	2.5
35	1528	1533	(*E*)-cadina-1,4-diene	0.1	tr	0.1	0.1	0.2	0.2	tr	tr	0.2
36	1538	1544	α-calacorene	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.2	0.3
37	1556	1555	elemicin	0.5	0.4	0.4	0.5	0.4	0.4	0.4	0.3	0.4
38	1561	1561	(*E*)-nerolidol	0.6	0.6	0.3	0.6	0.6	0.6	0.6	0.6	0.6
39	1573	1577	spathulenol	0.8	0.9	0.7	0.8	0.5	0.5	1.0	0.9	0.5
40	1578	1582	caryophyllene oxide	1.7	2.5	1.6	1.7	1.3	1.4	2.3	3.2	1.4
41	1586	1592	viridiflorol	0.9	0.9	0.9	0.9	0.9	0.9	0.9	1.2	0.9
42	1600	1600	ledol	0.2	tr	tr	0.2	0.1	tr	tr	0.2	tr
43	1604	1608	humulene epoxyde II	0.2	0.3	0.2	tr	0.1	0.1	0.3	0.5	0.1
44	1619	1620	dillapiole	81.6	82.4	78.8	78.8	74.3	73.9	80.5	80.2	73.5
45	1643	1644	α-muurolol	tr	tr	0.1	0.1	0.1	0.1	0.1	0.1	tr
46	1650	1652	α-cadinol	0.1	tr	tr	tr	tr	tr	tr	tr	tr
47	1660	1660	*cis*-calamenen-10-ol	0.1	tr	tr	tr	tr	tr	tr	tr	tr
48	1668	1668	*trans*-calamenen-10-ol	tr	0.7	tr	tr	tr	tr	tr	tr	tr
49	1677	1677	apiole	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
			Total:	99.9	99.9	99.3	99.9	99.5	99.8	99.2	97.6	99.9
			Monoterpenes:	0.0	0.3	0.0	0.0	0.4	0.7	0.1	0.1	1.4
			Oxygenated monoterpenoids:	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0
			Sesquiterpenes:	8.5	6.5	11.4	10.8	15.3	16.2	8.7	5.8	16.0
			Oxygenated sesquiterpenoids:	5.4	6.3	4.5	5.5	4.6	4.2	5.7	7.2	4.1
			Others:	86.0	86.8	83.4	83.6	79.2	78.8	84.7	84.5	78.4

LRI_{calc} = linear retention indices calculated according to Van den Dool and Kratz (1963) for a DB-5 stationary phase. LRI_{lit} = linear retention indices from the literature (Adams 2007; Joulain and Koenig 1998). UVCA = sample under UV light in an amber flask; UVCC = sample under UV light in a colorless flask; INAF = sample kept indoors at ambient temperature in an amber flask; INCF = sample kept indoors at ambient temperature in a colorless flask; REFA = sample kept in a refrigerator at 6 ºC in an amber flask; REFC = sample kept in a refrigerator at 6 ºC in a colorless flask. SUNA = sample kept under sunlight exposition (6 hours per day) in an amber flask; SUNC = sample kept under sunlight exposition (6 hours per day) in a colorless flask; CONT = sample stored in a domestic refrigerator, amber glass flask covered with aluminium foil and tr = trace (< 0.1 in área %).

Some studies have described loss of insecticidal efficacy of essential oils after storage. For example, essential oil of camphor, *Cinnamomum camphora* (L.) J. Presl and thyme, *Thymus serpyllum* L. stored in the presence of light and oxygen showed a toxic effect against larvae of *Aedes aegypti* L. for only two weeks (Amer and Mehlhorn 2006), indicating rapid degradation of the oxygenated monoterpenes when exposed to light (Misharina *et al*. 2003).

The minor changes observed in oil composition throughout time did not impact negatively the toxicity of EOPA. The stability of the arylpropanoids was enough to maintain the toxicity, as the observed losses of monoterpenes during the 4-year storage did not contribute to the occurrence

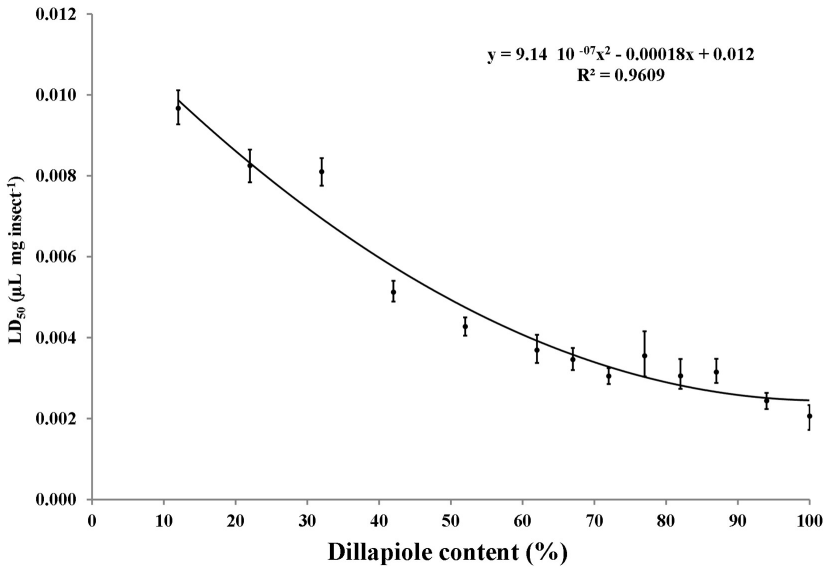

Figure 4. Toxicological effect by topical contact of fractions of *Piper aduncum* essential oil containing different dillapiole concentrations on *Spodoptera frugiperda* larvae. Points are the mean of four replicates and bars the 95% CI obtained by Probit analysis. R^2 = coefficient of determination.

of an additive effect on toxicity. Similarly, oils from *Piper* with higher content of arylpropanoids were more active against larvae of *A. aegypti* (Morais *et al*. 2007)

Terpenoids have an insecticidal effect by inhibiting acetylcholinesterase, which is associated with a neurotoxic effect in insects (da Silva *et al*. 2017). Likewise, the increase of sesquiterpenes such as germacrene D may have an insecticidal effect, as this compound is an inhibitor of esterases and glutathione S-transferases (Ribeiro 2012), which could increase lethality in synergy with other compounds present in the EOPA.

Dillapiole concentrations above 88% were less effective against *S. frugiperda* larvae then the medium-high concentrations, regardless of the route of contamination. This can be an advantage in the sense of simplifying the process to obtain a commercial product. This could be a result of a synergistic or additive interaction of minor compounds in the composition of EOPA with the dillapiole, contributing to the insecticidal effect, as has been observed in other studies on the evaluation of essential oils on insect control (Hummelbrunner and Isman 2001; Liu 2006; Gillij *et al*. 2008; Pavela 2008; Singh *et al*. 2009; Pavela 2014).

As dillapiole was the major compound of EOPA throughout the storage period, the retention of toxicity may be associated with a higher insecticidal activity of arylpropanoids, as pointed out by da Silva *et al*. (2017). Furthermore, the occurrence of other arylpropanoids in EOPA in addition to dillapiole, such as myristicin and elemicin, throughout the storage period, enables the inhibition of the three main families of detoxifying enzymes (P450 monooxygenases, esterases and glutathione S-transferases), allowing the increase in lethality of EOPA via synergistic interaction among its compounds (Bernard *et al*. 1993; Shankarganesh *et al*. 2009; Liu *et al*. 2014). *Piper* oil with dillapiole as major component (54.7%) had similar larvicidal activity against *A. aegipty* compared to enriched oil (98.9%) – 36.0 ppm and 42.9 ppm, respectively (Navarro *et al*. 2013). This may suggest a more important role in the definition of additivity or synergy in interactions between the major arylpropanoids present in EOPA.

Regardless of the contamination pathway, the larvicidal activity of EOPA mediated by dillapiole against *S. frugiperda* was optimal at concentrations of 82%–88%, which is the range indicated for the standardization in the prospection of future commercial products. Batch standardization may be achieved by indirect determination of the dillapiole content of EOPA through its refractive index, as proposed by Pateira *et al*. (1999), to control the limits for maximum insecticidal activity.

CONCLUSIONS

We established the relation between the dillapiole content and the insecticidal activity of EOPA against *S. frugiperda* larvae, and its chemical and toxicological stability when submitted to different storage conditions. EOPA dillapiole content was stable and its chemical composition did not vary significantly among different storage conditions over four years. The toxicological effects of EOPA on *S. frugiperda* larvae by topical and residual contact were not altered during this storage period. Dillapiole contents in the EOPA between 68% - 88% and 82% - 100% promote, respectively, optimal and stable residual and topical contact toxicity against *S. frugiperda* larvae. Further experiments should determine the efficacy of EOPA against multiple insect species, in order to generalize the trends of the observed phenomenon. Our results suggest that

EOPA can be employed as an eco-friendly natural alternative for chemical insecticides against *S. frugiperda*.

ACKNOWLEDGMENTS

The authors would like to thank Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Ministério da Agricultura, Pecuária e Abastecimento (MAPA-Brazil) for their financial support.

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RECEIVED: 16/08/2021
ACCEPTED: 24/05/2022
ASSOCIATE EDITOR: Jorge Mauricio David

SUPPLEMENTARY MATERIAL (only available in the electronic version)

Fazolin *et al*. Insecticidal activity of *Piper aduncum* oil: variation in dillapiole content and chemical and toxicological stability during storage

Table S1. Constituents of the essential oil of *Piper aduncum* from Acre state (Brazil) after one year of storage under different conditions (in area %).

Peak	LRIcalc	LRIlit	Identification	UVCA	UVCC	INAF	INCF	REFA	REFC	SUNA	SUNC	CONT
1	932	932	α-pinene	0.5	0.4	0.5	0.4	0.6	0.6	0.3	0.4	0.7
2	976	974	β-pinene	1.0	0.9	1.0	0.9	1.2	1.2	0.6	0.8	1.2
3	1005	1002	α-phellandrene	0.2	0.2	0.2	0.2	0.3	0.3	0.1	tr	0.4
4	1023	1020	*p*-cymene	0.1	0.1	0.1	0.1	tr	tr	tr	tr	0.1
5	1027	1024	limonene	0.2	0.2	0.2	0.2	0.4	0.3	0.1	tr	0.3
6	1036	1032	(*Z*)-β-ocimene	0.2	0.1	0.2	0.2	0.2	0.2	tr	tr	0.3
7	1046	1044	(*E*)-β-ocimene	0.4	0.3	0.4	0.3	0.5	0.5	0.2	tr	0.6
8	1365	1369	cyclosativene	0.2	tr	0.2	0.2	tr	tr	0.2	tr	tr
9	1374	1374	α-copaene	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1
9A	1383	1387	β-bourbonene	tr	tr	tr	tr	tr	tr	tr	0.4	tr
10	1391	1389	β-elemene	0.1	0.1	0.1	0.1	tr	tr	0.2	0.2	tr
11	1418	1417	(*E*)-caryophyllene	8.6	8.4	8.5	8.6	8.9	9.0	8.5	8.1	9.0
12	1436	1439	α-trans-bergamotene + aromadendrene	0.3	tr	0.3	0.3	tr	tr	tr	tr	tr
13	1451	1452	α-humulene	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1
14	1459	1457	β-santalene	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.4	0.5
15	1479	1459	allo-aromadendrene	0.9	0.7	0.9	0.9	1.0	1.1	0.8	tr	1.1
16	1494	1494	*epi*-cubebol + bicyclogermacrene	0.9	0.8	0.9	0.9	1.1	1.1	0.9	0.8	1.1
17	1500	1500	pentadecane	1.7	1.7	1.7	1.8	1.7	1.7	1.9	1.9	1.7
18	1506	1511	δ-amorphene	0.3	0.2	0.2	0.2	0.4	0.4	0.2	tr	0.4
19	1514	1514	cubebol	0.8	0.8	0.7	0.8	0.8	0.8	0.8	0.8	0.8
20	1522	1522	miristicin + δ-cadinene	2.6	2.6	2.6	2.6	2.6	2.6	2.7	2.6	2.6
21	1541	1544	α-calacorene	0.3	tr	0.3	0.3	tr	tr	0.3	tr	tr
22	1559	1555	elemicin	0.2	0.2	0.2	0.2	tr	tr	0.2	tr	tr
23	1564	1561	(*E*)-nerolidol	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
24	1576	1577	spathulenol	0.7	0.7	0.6	0.7	0.4	0.5	0.7	0.7	0.4
25	1581	1582	caryophyllene oxide	1.5	1.7	1.5	1.6	1.2	1.2	1.7	1.9	1.2
26	1589	1592	viridiflorol	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9
27	1607	1608	humulene epoxyde II	0.2	tr	0.2	0.2	tr	tr	tr	tr	tr
28	1632	1620	dillapiole	74.2	75.8	74.4	74.7	74.5	74.5	75.8	77.5	74.1
			Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
			Monoterpenes	2.7	2.2	2.7	2.2	3.3	3.2	1.4	1.2	3.5
			Oxygenated monoterpenoids	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
			Sesquiterpenes	14.3	12.9	14.1	14.1	14.2	14.2	13.7	12.1	14.4
			Oxygenated sesquiterpenoids	4.4	4.5	4.4	4.5	3.7	3.7	4.5	4.7	3.7
			Others	78.7	80.3	78.9	79.3	78.8	78.8	80.4	82.0	78.5

LRI_{calc} = linear retention indices calculated according to Van den Dool and Kratz (1963) for a DB-5 stationary phase. LRI_{lit} = linear retention indices from the literature (Adams 2007; Joulain and Koenig 1998). UVCA = sample under UV light in an amber flask; UVCC = sample under UV light in a colorless flask; INAF = sample kept indoors at ambient temperature in an amber flask; INCF = sample kept indoors at ambient temperature in a colorless flask; REFA = sample kept in a refrigerator at 6 ºC in an amber flask; REFC = sample kept in a refrigerator at 6 ºC in a colorless flask. SUNA = sample kept under sunlight exposition (6 hours per day) in an amber flask; SUNC = sample kept under sunlight exposition (6 hours per day) in a colorless flask; CONT = sample stored in a domestic refrigerator, amber glass flask covered with aluminium foil and tr = trace (< 0.1 in área %).

Table S2. Constituents of the essential oil of *Piper aduncum* from Acre state (Brazil) after two years of storage under different conditions (in area %).

Peak	LRIcalc	LRIlit	Identification	UVCA	UVCC	INAF	INCF	REFA	REFC	SUNA	SUNC	CONT
1	932	932	α-pinene	tr	0.1	0.1	0.2	0.6	0.5	tr	tr	0.6
2	975	974	β-pinene	0.2	0.3	0.4	0.5	1.1	1.0	0.1	0.1	1.2
3	1004	1002	α-phellandrene	tr	tr	tr	tr	0.3	0.3	tr	tr	0.3
4	1022	1020	*p*-cymene	tr	tr	tr	tr	0.1	0.1	tr	tr	
5	1026	1024	limonene	tr	tr	tr	tr	0.2	0.2	tr	tr	0.2
6	1035	1032	(*Z*)-β-ocimene	tr	tr	tr	tr	0.2	0.2	tr	tr	0.2
7	1045	1044	(*E*)-β-ocimene	tr	tr	tr	tr	0.4	0.3	tr	tr	0.3
8	1345	1345	α-cubebene	tr	tr	tr	tr	tr	tr	tr	0.1	tr
9	1362	1369	cyclosativene	tr	tr	tr	0.2	0.2	0.2	0.1	0.1	0.2
10	1371	1374	α-copaene	0.7	0.8	1.0	1.0	1.1	1.1	0.9	0.9	1.1
11	1379	1387	β-bourbonene	tr	tr	tr	tr	tr	tr	tr	0.3	tr
12	1388	1389	β-elemene	tr	tr	0.1	0.2	0.3	0.3	0.2	0.2	0.1
13	1414	1417	(*E*)-caryophyllene	6.9	6.8	8.3	8.2	8.9	8.9	7.5	4.4	9.1
14	1423	1430	β-copaene	tr	tr	tr	tr	0.1	0.1	tr	0.2	tr
15	1433	1432	*trans*-α-bergamotene	tr	tr	tr	0.3	0.3	0.3	tr	0.3	tr
16	1448	1452	α-humulene	1.0	1.0	1.1	1.1	1.1	1.1	1.0	0.8	1.2
17	1455	1457	β-santalene	tr	0.4	0.4	0.4	0.5	0.5	0.4	0.4	0.5
18	1475	1484	germacrene D	0.8	0.5	0.9	0.8	1.0	1.0	0.6	tr	1.1
19	1479	1479	ar-curcumene	tr	tr	tr	tr	tr	tr	tr	0.2	tr
20	1491	1494	bicyclogermacrene	tr	0.6	0.9	0.8	1.1	1.1	0.1	0.4	1.1
21	1496	1500	pentadecane	1.2	1.5	1.5	1.7	1.6	1.6	1.5	1.6	1.7
22	1502	1511	δ-amorphene	tr	tr	0.2	0.1	0.4	0.4	tr	tr	0.4
23	1510	1513	γ-cadiene	0.7	0.7	0.8	0.8	0.8	0.8	0.7	0.5	0.8
24	1518	1517	miristicin	1.8	2.1	2.2	2.3	2.3	2.3	2.3	2.4	2.1
25	1527	1533	*trans*-cadina-1,4-diene	tr	tr	tr	tr	0.1	tr	tr	tr	tr
26	1537	1544	α-calacorene	tr	tr	0.3	0.3	0.3	0.3	0.3	0.2	tr
27	1546		n.i.	tr	tr	tr	tr	tr	tr	tr	0.2	tr
28	1555	1555	elemicin	tr	tr	tr	0.1	0.1	0.1	tr	0.2	tr
29	1560	1561	(*E*)-nerolidol	tr	0.4	0.4	0.5	0.4	0.4	0.4	0.5	0.4
30	1572	1577	spathulenol	0.7	0.8	0.7	0.8	0.6	0.6	0.9	1.0	0.5
31	1577	1582	caryophyllene oxide	1.7	2.1	1.6	1.7	1.3	1.3	2.1	3.3	1.3
32	1585	1592	viridiflorol	1.0	1.0	0.9	0.9	0.9	0.9	1.0	1.0	0.9
33	1603	1608	humulene epoxyde II	tr	tr	tr	0.2	0.1	0.2	0.2	0.4	tr
34	1618	1620	dillapiole	83.3	80.8	78.1	76.8	73.6	74.2	79.2	80.2	74.8
35	1655		n.i.	tr	tr	tr	tr	tr	tr	tr	tr	tr
36	1666		n.i.	tr	tr	tr	tr	tr	tr	tr	0.2	tr
37	1994		n.i.	tr	tr	tr	tr	tr	tr	0.3	tr	tr
38	2014		n.i.	tr	tr	tr	tr	tr	tr	tr	tr	tr
			total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
			Monoterpenes	0.0	0.4	0.5	0.7	2.9	2.5	0.1	0.1	2.8
			Oxygenated monoterpenoids	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
			Sesquiterpenes	10.1	10.8	14.1	14.2	16.2	16.0	12.0	9.0	15.5
			Oxygenated sesquiterpenoids	3.4	4.3	3.6	4.1	3.3	3.3	4.5	6.2	3.0
			Others	86.3	84.4	81.8	81.0	77.6	78.2	83.1	84.5	78.7

LRI_{calc} = linear retention indices calculated according to Van den Dool and Kratz (1963) for a DB-5 stationary phase. LRI_{lit} = linear retention indices from the literature (Adams 2007; Joulain and Koenig 1998). UVCA = sample under UV light in an amber flask; UVCC = sample under UV light in a colorless flask; INAF = sample kept indoors at ambient temperature in an amber flask; INCF = sample kept indoors at ambient temperature in a colorless flask; REFA = sample kept in a refrigerator at 6 ºC in an amber flask; REFC = sample kept in a refrigerator at 6 ºC in a colorless flask. SUNA = sample kept under sunlight exposition (6 hours per day) in an amber flask; SUNC = sample kept under sunlight exposition (6 hours per day) in a colorless flask; CONT = sample stored in a domestic refrigerator, amber glass flask covered with aluminium foil and tr = trace (< 0.1 in área %).

Table S3. Constituents of the essential oil of *Piper aduncum* from Acre state (Brazil) after three years of storage under different conditions (in area %).

Peak	LRIcalc	LRIlit	Identification	UVCA	UVCC	INAF	INCF	REFA	REFC	SUNA	SUNC	CONT
1	931	932	α-pinene	0.0	0.0	0.0	0.0	0.6	0.5	tr	tr	0.5
2	946	946	canphene	tr	tr	tr	tr	0.0	tr	tr	tr	tr
3	975	974	β-pinene	0.1	0.1	0.1	0.1	1.1	1.1	tr	tr	1.1
4	1004	1002	α-phellandrene	tr	tr	tr	tr	0.3	0.3	tr	tr	0.3
5	1023	1020	*p*-cymene	tr	tr	tr	tr	0.1	0.1	tr	tr	tr
6	1026	1024	limonene	tr	tr	tr	tr	0.3	0.2	tr	tr	0.2
7	1035	1032	(*Z*)-β-ocimene	tr	tr	tr	tr	0.2	0.2	tr	tr	tr
8	1045	1044	(*E*)-β-ocimene	tr	tr	tr	tr	0.3	0.3	tr	tr	tr
9	1345	1345	α-cubebene	tr	0.1	tr	tr	0.1	tr	0.1	0.1	tr
10	1362	1369	cyclosativene	0.1	0.1	0.1	0.1	0.2	0.2	0.1	0.1	0.2
11	1371	1374	α-copaene	0.6	0.6	0.9	0.9	1.1	1.1	0.7	0.9	1.1
12	1380	1387	β-bourbonene	tr	tr	tr	tr	tr	tr	tr	0.3	tr
13	1386	1387	β-cubebene	0.1	tr	0.1	tr	0.1	0.1	tr	tr	tr
14	1388	1389	β-elemene	0.2	0.1	0.2	0.2	0.2	0.2	0.2	0.2	0.1
15	1404	1409	α-gurjunene	tr	tr	tr	tr	0.1	tr	tr	0.1	tr
16	1415	1417	(*E*)-caryophyllene	5.9	5.2	7.7	7.3	8.9	8.9	6.2	2.7	9.1
17	1424	1430	β-copaene	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.2	tr
18	1432	1432	*trans*-α-trans-bergamotene	tr	tr	tr	tr	0.1	tr	tr	tr	tr
19	1433	1439	aromadendrene	0.2	0.2	0.3	0.3	0.2	0.3	0.2	0.3	tr
20	1448	1452	α-humulene	0.9	0.8	1.0	1.0	1.1	1.1	0.9	0.6	1.1
21	1455	1457	β-santalene	0.4	0.4	0.4	0.4	0.5	0.5	0.4	0.4	0.5
22	1472	1478	γ-muroleno	0.1	0.1	0.1	0.1	0.1	0.1	0.1	tr	tr
23	1476	1484	germacrene D	0.7	0.3	0.8	0.7	1.0	1.0	0.4	tr	1.0
24	1480	1479	ar-curcumene	0.1	tr	0.1	0.2	tr	tr	0.2	0.3	tr
25	1486	1493	*trans*-muurola-4-(14), 5-diene	0.1	0.2	0.1	tr	0.1	tr	tr	tr	tr
26	1491	1494	bicyclogermacrene	0.7	0.5	0.8	0.7	1.1	1.0	0.5	0.4	1.1
27	1497	1500	pentadecane	1.3	1.3	1.5	1.5	1.6	1.6	1.2	1.5	1.7
28	1502	1511	δ-amorphene	0.1	tr	0.1	tr	0.4	0.4	tr	tr	0.4
29	1505	1505	α-(*E,E*)-farnesene	tr	tr	tr	tr	0.1	tr	tr	tr	tr
30	1509		n.i.	0.2	tr	0.1	tr	0.2	0.2	tr	tr	tr
31	1511	1513	γ-cadinene	0.6	0.6	0.6	0.7	0.6	0.6	0.7	0.6	0.5
32	1519	1517	miristicin	2.3	2.3	2.4	2.3	2.3	2.1	2.3	2.1	2.0
33	1528	1533	*trans*-cadina 1,4 diene	0.1	tr	0.1	0.1	0.1	0.1	tr	tr	tr
34	1538	1544	α-calacorene	0.3	0.3	0.3	0.3	0.3	0.3	0.3	tr	0.3
35	1551	1559	germacrene B	0.1	0.1	0.1	0.1	0.1	0.1	0.3	0.2	tr
36	1556	1555	elemicin	0.1	0.1	0.1	0.2	0.3	0.1	0.2	0.2	tr
37	1561	1561	(*E*)-nerolidol	0.4	0.5	0.4	0.6	0.5	0.4	0.6	0.5	0.4
38	1573	1577	spathulenol	0.8	0.9	0.7	0.8	0.5	0.6	0.9	0.9	0.6
39	1578	1582	caryophyllene oxide	1.6	2.2	1.6	1.7	1.3	1.3	2.2	3.4	1.3
40	1586		n.i.	0.9	0.9	0.9	0.9	0.8	0.9	0.9	1.2	0.9
41	1597		n.i.	0.2	0.1	0.2	tr	0.2	tr	0.2	0.2	tr
42	1603	1608	humulene epoxyde II	0.2	0.2	0.2	0.2	0.1	0.1	0.2	0.5	tr
43	1607		n.i.	0.1	tr	tr	tr	tr	tr	tr	tr	tr
44	1619	1620	dillapiole	80.5	81.6	77.6	78.5	72.7	74.0	79.8	82.0	75.7
45	1644		n.i.	0.1	0.1	0.1	tr	0.1	tr	0.1	tr	tr
			Total	98.5	96.8	98.7	99.1	98.8	99.0	98.8	98.6	99.1
			Monoterpenes	0.2	0.2	0.2	0.2	2.9	2.6	0.0	0.0	2.1
			Otrygenated monoterpenoids	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
			Sesquiterpenes	11.2	9.5	14.1	13.2	16.6	16.1	11.4	7.5	15.3
			Otrygenated sesquiterpenoids	3.0	3.8	2.9	3.2	2.5	2.4	3.9	5.3	2.3
			Others	85.6	86.5	82.9	83.4	78.1	78.8	84.7	87.2	80.3

LRI_{calc} = linear retention indices calculated according to Van den Dool and Kratz (1963) for a DB-5 stationary phase. LRI_{lit} = linear retention indices from the literature (Adams 2007; Joulain and Koenig 1998). UVCA = sample under UV light in an amber flask; UVCC = sample under UV light in a colorless flask; INAF = sample kept indoors at ambient temperature in an amber flask; INCF = sample kept indoors at ambient temperature in a colorless flask; REFA = sample kept in a refrigerator at 6 ºC in an amber flask; REFC = sample kept in a refrigerator at 6 ºC in a colorless flask. SUNA = sample kept under sunlight exposition (6 hours per day) in an amber flask; SUNC = sample kept under sunlight exposition (6 hours per day) in a colorless flask; CONT = sample stored in a domestic refrigerator, amber glass flask covered with aluminium foil and tr = trace (< 0.1 in área %).