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BIOINSECTICIDE POTENTIAL OF *Curcuma zedoaria* RHIZOME ESSENTIAL OIL

POTENCIAL BIOINSETICIDA DO ÓLEO ESSENCIAL DOS RIZOMAS DE Curcuma zedoaria

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ABSTRACT: In this study the potential bioinseticide of the essential oil (OE) extracted from the rhizomes of the species *Curcuma zedoaria* (Zingiberaceae) was evaluated. The rhizomes were collected during dormancy (winter) and budding (summer). The EO was obtained by hydrodistillation (2h) and identified by GC/MS. In addition, a multivariate exploratory analysis was done to determine the analysis of the major compounds (PCA). The EO yield in dormancy was 0.61± 0.07 (%) and in budding 0.55 ± 0.08 (%). The bioassays on *Aedes aegypti* larvae and pupae were done by immersion test at different EO concentrations which ranged from 500.00 to 0.003 mg mL^{-1} (v/v). The results on the larvae and pupae indicated $LC_{99.9}$ of (0.01 and 1.38 mg mL^{-1}) for EO in dormancy, and (0.08 and 2.63 mg mL^{-1}) for EO during budding, respectively. The action mechanism of EOs in both periods was determined by autobiographic method evaluating the inhibitory potential on the acetylcholinesterase enzyme, indicating greater inhibition of the EO enzyme during dormancy (0.039 mg mL^{-1}) when compared to the EO during budding (0.156 mg mL^{-1}). The projection representation of the EO chemical classes in both evaluated periods indicated that oxygenated sesquiterpenes are the major compound class (46.99% in dormancy) and (43.59% in budding). The projection of major chemical compounds of EOs presented three compounds with greater mass flow distancing: epicurzerenone (18.20% and 12.10%); 1.8 cineole (15.76% and 12.10%) and *β*-elemene (4.43 and 0.01%) that are found in greater amounts in the dormancy EO when compared to budding, respectively. These results corroborate with the greater potential on *Ae. aegypti* larvae and pupae found for the dormancy EO. The results are promising because they show in which vegetative cycle phase *C. zedoaria* EO presents greater bioinsecticide potential.

KEYWORDS: *Aedes aegypti*. Anticholinesterase. 1,8 Cineole. Epicurzerenone. *β*-Elemene. Lethal concentration (LC).

ABBREVIATIONS: CG/MS gas chromatography coupled to mass spectrometer; LC lethal concentration; $LC_{99.9}$ lethal concentration to eliminate 99.9% of larvae and pupa; EO essential oil.

INTRODUCTION

The species *Aedes aegypti* L. is the main vector transmitting Dengue, Chikungunya fever and Zika virus, which are responsible for significant human morbidity and mortality in a lot of countries, (BRASIL, 2015; WORLD HEALTH ORGANIZATION (WHO), 2016).

According to the epidemiologic report of the Health Ministry- from December 31, 2017 to March 10, 2018) - 51,980 cases of dengue, 12,261 cases of Chikungunya fever were reported, and in 2018 there must have been 1,174 probable cases of fever by Zika virus (BRASIL, 2018). In Brazil, the high incidence of these transmitting insects is due to the accelerated and disorderly urbanization process as well as the climatic and social conditions that favor their quick proliferation (OLIVEIRA; BIAZOTO, 2012; BUSATO et al., 2014).

The strategies to eradicate *Ae. aegypti* consist of the application of synthetic insecticides, mainly organophosphates and pyrethroids (BRAGA; VALLE, 2007). However, the utilization of these insecticides must be careful to avoid slowing down the development

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of resistance to them (SMITH; KASAI; SCOTT, 2016), toxicity to humans as well as the impact that they cause to biodiversity (NICOLAU, 2013). Therefore, research studies utilizing biomolecules such as plant essential oils have been done (COSMOSKI et al., 2015) because these chemical structural groupings of these compounds or their combination can have, and also intensify, the larvicidal potential (SANTOS et al., 2010).

Considering this, the evaluated plant, *Curcuma zedoaria* (Zingiberaceae), is an exotic plant from Southern and southeastern Asia, which is well-adapted to the Southern region of Brazil, especially the northeastern region of Paraná state. Popularly known as zedoaria, vick and fake saffron, this species has been studied due to its therapeutic potential with several scientific results that confirm its traditional and popular medicinal use (LORENZI; 2002).

Its cultivation is easy and its rhizomes are rich in essential oil, mainly consisting of pinene, camphene, cineol, camphor and borneol (GUENTHER, 1950). However, this composition varies and is influenced by the plant vegetative phase and abiotic factors such as radiation, temperature, rainfall, winds, altitude, soil and cultivation implementation location (MORAIS, 2000; GOUINGUENÉ; TURLINGS, 2002). Research studies on its EO larvicidal potential are scarce and, therefore, caused our research group to deepen the investigations on the probable action against *Ae. aegypti* larvae and pupae.

Thus, this study aimed to evaluate the larvicidal potential of *Curcuma zedoaria* essential oil obtained in budding and dormancy periods against *Ae. aegypti* larvae and pupae.

MATERIAL AND METHODS

Botanical identification and vegetal material preparation

The cultivation of *Curcuma zedoaria* is found in the Medicinal Garden of the Paranaense University – Umuarama – PR. An exsiccata is deposited in the Educational Herbarium of Paranaense University – HEUP, under the registration number 2400. The botanical identification was done by Prof. Dr. Ezilda Jacomassi. This species is registered in the National System for the Management of Genetic Heritage and Associated Traditional Knowledge (SisGen) under the registration number ACFC9FF

The vegetal material was dried, powdered and submitted to hydrodistillation process for two hours. The oil was withdrawn with a Pasteur pipette, filtered with Na_2SO_4 and stored at -10ºC (LAI et al., 2004).

Chemical composition analysis of *Curcuma zedoaria* essential oil

The chemical composition analysis was done using a gas chromatographer (Agilent 7890 B) coupled to mass spectrometer (Agilent 5977 A) equipped with an Agilent HP-5MS (30m x 0.250mm x 0.25mμ) under the following conditions: injector temperature at 250°C, injection volume 1mL at a 1:2 rate (splitless mode), initial temperature of the column at 80°C with gradual heating until 260°C and a ramp of 4°C/min. The carrier gas (helium) flow was fixed at 1 mL/min. The transfer line temperatures and quadrupole ion sources were 250, 230, and 150°C, respectively. The mass spectra were obtained in a 40-500 (m/z) interval provided by scanning with solvent permanence time of 3 min. The compounds were identified by comparing their retention indices (RI) obtained from a series of n-alkanes (C8-C25) (LAI et al., 2004). Moreover, EI-mass spectra were compared to the spectra obtained from Wiley's Spectral Library 275L, and according to the literature (ADAMS, 2012).

Major Compound Analysis (PCA)

Complementarily, a multi-varied exploratory analysis was also done to determine the main compound analysis (PCA) which allowed the evaluation of the major chemical compounds and chemical class of all compounds found in the essential oil obtained in three analyzed periods (vegetative, flowering and fructification). The analysis result was graphically presented (Biplot), helping the characterization of the analyzed variable groups (MOITA NETO; MOITA, 1998).

For each sample of essential oil obtained during these three periods (vegetative, flowering and fructification), the identified major chemical compounds and their respective chemical classes, as well as the area amount in (%) (Table 1), were plotted in Excel spreadsheets. These data were transformed in orthogonal latent variables called major components that are linear combinations of original variables created with the eigenvalues of the data covariance matrix (HAIR, 2005).

Kaiser's criterion was utilized to choose the main components. An eigenvalue preserves

the relevant information when it is greater than the unit. This analysis was carried out in two ways: the former contained only data referring to the chemical composition of the major compounds obtained in the three periods, and the latter analyzed the grouped chemical classes to which those compounds belong to. Both analyses were done utilizing Statistica 7 program (STATSOFT, 2001).

Larvicidal activity against *Aedes aegypti*

The larvicidal activity was done by Larval Immersion Test (LIT) against *Aedes aegypti* L. larvae and pupae from the Center for Vector transmitted Endemics – Secretary of Sanitary Surveillance of Umuarama, PR. The EO was tested at different concentrations: 500, 400, 300, 200, 100, 50, 25, 12.5, 6.25, 3.125, 1.562, 0.781, 0.390, 0.195, 0.097, 0.048, 0.024, 0.012, 0.006, 0.003 mg mL^{-1} (v/v), diluted in an aqueous polysorbate 80 solution at 2.0%. Five third-stage *Ae. aegypti* larvae and five pupae were separated using a Pasteur pipette and placed in assay tubes containing 1.00 mL of different concentrations of EO (COSTA et al., 2005; CAVALCA; LOLIS BONATO, 2010). For the negative control, an aqueous polysorbate 80 solution at 2% was utilized whereas for the positive control, a commercial Temephós® solution at a concentration of 400 mg L^{-1} was used (CAMARGO et al., 1998). The larvae were exposed to EO at different concentrations for 24 hours (CARVALHO et al., 2003). They were considered dead when presented absence of movements and were irresponsive to stimuli (CAVALCA; LOLIS; BONATO, 2010). The data on the number of live and dead larvae and pupae were found through an average of three replications for each one of the tested concentrations.

Anticholinesterase activity of essential oil

The anticholinesterase activity was determined by the bioautographic method described by Marston et al. (2002) with modifications (YANG et al., 2009). The essential oil of *C. zedoaria* rhizome was tested starting from the initial concentration of 10, 5, 2.5, 1.25, 0.625, 0.3125, 0.1562, 0.078, 0.039, 0.019 mg mL^{-1}, diluted in methanol. The samples were plotted in aluminum chromo plates (10 x 10 cm, 0.2 mm-thick 60 F254 silica gel). After plotting, the plates were dried and sprayed with an acetylcholinesterase enzyme solution in buffer solution; next, they were sprayed with an α-naftyl acetate solution. The plates were kept at 37 °C for 20 minutes. After this period, the chromo plates were sprayed with Fast Blue B salt colorimetric reagent, resulting in a purple color surface. The anticholinesterase activity of *C. zedoaria* rhizome EO was determined by the emergence of white stains after 10 minutes, showing the inhibitory action of the evaluated concentrations on the enzyme activity, contrasting with the purple color of the colorimetric reagent (COLLINS et al., 1997) like the positive standard of the larvicide (Temephós®).

Statistical analysis

The experiments were done in triplicate and the mortality percentage (%) of *Ae. aegypti* larvae was obtained by calculating the mean ± standard deviation and coefficient variation utilizing Microsoft Excel® program (Excel® Version 2010). The values of Lethal Concentration (LC_{50} and $LC_{99.9}$) and their respective confidence intervals (CI) were calculated by Probitos analysis (ED 50 Plus version 1.0). The obtained data were submitted to analysis of variance (ANOVA) and the differences between the averages were determined by Scott-Knott's test ($p \leq 0.05$).

RESULTS AND DISCUSSION

The essential oil of *Curcuma zedoaria* rhizome has lilac coloring, with greater yield in the dormancy period (0.61 ± 0.07 %) compared to the budding period (0.55 ± 0.08 %). This difference in the yield is justified because in this period the buds are in the peak of their maturation (DING; NILSSON, 2016), and the found values are in accordance with Angel et al. (2014), who found yields ranging from 0.38 to 1.4 (%) for *C. zedoaria* species.

The chemical identification of the essential oil obtained during the dormancy and budding periods was done by a gas chromatographer coupled to a mass spectrometer and whose results are shown in Table 1 and Figures 1 and 2.

Table 1. Chemical composition of essential oils obtained from *Curcuma zedoaria* rhizomes during dormancy and vegetative periods.

Peak	[a]Compound	[b]RI	Dormancy	Budding	Peak	[a]Compound	[b]RI	Dormancy	Budding	Identification Methods
			Relative Area (%)					Relative Area (%)		
1	Heptanol	880	t	0.03	**20**	*trans*-sabinene	1068	0.33	0.22	a,b,c
2	Tricyclene	899	0.06	-	**21**	*β*-thujone	1079	-	0.53	a,b,c
3	*α*- thujene	905	0.05	0.08	**22**	*α*- thujone	1088	t	0.04	a,b,c
4	*α*- pinene	907	0.85	0.06	**23**	*cis*-limonene oxide	1095	0.05	-	a,b,c
5	Fenchene	913	-	0.82	**24**	*cis*- vebenol	1115	t	0.07	a,b,c
6	Camphene	929	1.39	1.38	**25**	Camphor	1117	5.61	5.83	a,b,c
7	Sabinene	956	0.27	-	**26**	Camphene hydrate	1118	0.06	t	a,b,c
8	*β*-pinene	958	1.55	1.79	**27**	Isoborneol	1126	1.51	1.83	a,b,c
9	Myrcene	973	0.41	0.50	**28**	Pinocarvone	1130	t	0.05	a,b,c
10	*δ*- carene	984	t	0.04	**29**	Borneol	1133	0.6	0.86	a,b,c
11	*α*-phellandrene	989	t	0.08	**30**	Terpineol	1144	0.45	0.68	a,b,c
12	*α*- terpine	996	0.05	0.07	**31**	*α*- terpineol	1156	1.03	1.37	a,b,c
13	*p*-cymene	1005	t	-	**32**	Myrtenol	1160	t	0.07	a,b,c
14	Limonene	1006	-	t	**33**	*cis* –piperitol	1163	t	0.09	a,b,c
15	1,8 cineole	1017	15.76	12.10	**34**	Verbenone	1170	-	0.08	a,b,c
16	n.i	1028	-	0.10	**35**	*β*- citronellol	1177	-	0.06	a,b,c
17	*cis*- sabinene hydrate	1045	t	0.13	**36**	*trans*- carveol	1186	t	0.19	a,b,c
18	Nonanone	1061	0.14	0.04	**37**	Citronellol	1196	-	0.22	a,b,c
19	Linalool	1064	t	0.07	**38**	*cis*- carveol	1197	0.11	0.03	a,b,c

39	Carvone	1209	0.15	0.02	**59**	*γ*- gurjunene	1477	-	0.06	a,b,c
40	Isogeraniol	1221	0.08	-	**60**	*γ*- selinene	1484	-	0.36	a,b,c
41	Myrtanol	1233	t	0.06	**61**	Germacrene D	1489	2.42	2.05	a,b,c
42	*trans*-geraniol	1239	-	0.10	**62**	*β*- selinene	1492	0.67	0.84	a,b,c
43	Undecanone	1241	-	0.13	**63**	*α*-selinene	1501	4.68	4.78	a,b,c
44	*cis -p*-mentha -6,8- dien-2-ol acetate	1265	-	0.03	**64**	Curzerene	1507	1.07	-	a,b,c
45	Myrtenyl acetate	1274	-	0.01	**65**	*α*- muurolene	1511	-	0.24	a,b,c
46	*δ*- elemene	1276	1.05	1.03	**66**	Bicyclogermacrene	1513	0.08	0.08	a,b,c
47	*α*-copaene	1302	0.29	0.01	**67**	*β*- guaiene	1519	0.34	0.55	a,b,c
48	*β*- elemene	1310	4.43	0.01	**68**	*β*- cadinene	1521	-	0.50	a,b,c
49	*α*- gurjunene	1320	0.20	1.09	**69**	*γ*- cadinene	1523	0.24	-	a,b,c
50	*γ*- caryophyllene	1420	-	0.42	**70**	*δ*- cadinene	1525	0.07	-	a,b,c
51	*β*-caryophyllene	1433	-	4.34	**71**	Himachalene	1532	-	0.43	a,b,c
52	*trans*-caryophyllene	1436	0.05	-	**72**	Selina 3,7 diene	1535	0.08	0.08	a,b,c
53	*γ*- elemene	1446	1.12	0.05	**73**	Germacrene B	1540	0.94	0.27	a,b,c
54	*α*-guaiene	1450	0.33	0.62	**74**	Aristolene	1545	-	0.17	a,b,c
55	Aromadrendene	1457	0.28	0.06	**75**	Longifolene	1547	-	0.94	a,b,c
56	*α*-humulene	1461	0.18	0.32	**76**	Sphatulenol	1552	0.15	0.24	a,b,c
57	*trans β*- farnesene	1470	3.14	3.06	**77**	Caryophyllene oxide	1555	0.12	0.23	a,b,c
58	Allo aromadrendene	1471	0.30	t	**78**	*α*- copaene- 8 ol	1564	-	0.34	a,b,c

79	*γ*- elemene epoxy	1567	0.27	-	**97**	Cedrenol	1643	-	0.16	a,b,c
80	Globulol	1568	0.61	-	**98**	Cedren 13 ol	1649	1.39	-	a,b,c
81	Viriflorol	1570	0.69	0.82	**99**	Germacrone	1653	3.29	3.33	a,b,c
82	*β*- elemone	1574	-	1.00	**100**	n.i	1659	0.29	-	a,b,c
83	Epicurzerenone	1591	18.20	14.05	**101**	Farnesol	1761	0.33	0.38	a,b,c
84	Caryophyllenol	1592	-	0.82	**102**	Valerenal	1768	0.35	-	a,b,c
85	Curzerenone	1602	0.54	2.17	**103**	n.i	1769	0.32	-	a,b,c
86	Caryophylla 3,8(13)- dien 5	1610	0.31	0.64	**104**	Elema 1,3 ,11(13) –trien- 12ol	1779	2.77	0.82	a,b,c
87	*α*- muurolol	1610	2.02	-	**105**	Lanceol	1791	1.01	1.09	a,b,c
88	*β*- eudesmol	1613	0.82	-	**106**	*α*- costol	1791	-	2.97	a,b,c
89	n.i	1618	0.45	-	**107**	Aromadrendene epoxy	1793	-	2.53	a,b,c
90	Valerenol	1621	0.79	0.38	**108**	2,3-Hexadienoic acid, 2-methyl-4-phenyl-, methyl ester	1794	-	1.56	a,b,c
91	*epi*- amiteol	1621	-	1.39	**109**	n.i	1801	0.51	0.30	a,b,c
92	n.i	1624	0.48	-	**110**	Azuleno [6,5-b] furan -2(3h)-	1818	3.56	3.09	a,b,c
93	*α*- santalol	1624	0.60	0.54	**111**	Isocurcumenol	1826	4.78	3.91	a,b,c
94	Juniper camphor	1630	-	0.34	**112**	n.i	1828	0.14	0.16	a,b,c
95	Vulgarol	1633	-	0.52	**113**	Allyl ionone	1843	-	0.24	a,b,c
96	n.i	1638	0.61	0.36	**114**	Anisole m octyl	1856	-	1.15	a,b,c
115	Solavetivone	1865	-	0.41						

116	2,3-Hexadienoic acid, 2-methyl-4-phenyl-, methyl ester	1876	-	0.10
117	Iso-α-cedren 14,15 dial	1879	-	0.20
118	n.i	1888	-	0.22
119	Isovelleral	1895	0.70	0.29
120	n.i	1909	-	0.86
121	Eudesma 1,4 dien -12 ol	1878	-	0.08
122	Cembrene	1987	t	0.05
123	2-(Fench-2-yl) fenchane	2917	-	0.04
	Total Identified		**95.77**	**98.00**
	Hydrocarbons Monoterpenes		4.63	4.95
	Oxygenated Monoterpenes		25.74	24.57
	Hydrocarbon Sesquiterpenes		21.96	22.36
	Oxygenated Sesquiterpenes		43.30	43.89
	Oxygenated Diterpenes		0.00	0.09
	Others compounds		0.14	2.14

[a]Compound listed in order of elution from HP-5 column; [b]IR: Retention index calculated using *n*-alkane C7 - C28 in HP-5 column; [c]IR: Identification based on retention index reported by Adams (2012) and identification based on comparison of mass spectra using NIST 11.0 library; Relative area (%): percentage of the area occupied by the compounds in the chromatogram; t= trace; n.i = not identified; (-): absent.

According to the data in Table 1, the rhizome EO from the dormancy period presented 120 compounds, and from the budding period 119 compounds. According to Figure 1 and Table 1, the predominant class was oxygenated Sesquiterpenes (43.30% and 43.89%), followed by oxygenated Monoterpenes (25.74 % and 24.57%) and hydrocarbon sesquiterpenes (21.96% and 22.36%), respectively. In Figure 2, grouping by PCA was done and major compounds were identified in EO in dormancy and budding. Three of these compounds presented a greater mass flow distancing : epicurzerenone with 18.20% in dormancy EO and 14.05% in budding EO, 1,8-cineole (15.76% and 12.10%) and *β*-elemene (4.43 and0.01%),respectively. Moreover, it was possible to verify the formation of other group: camphor (5.61 and 5.83), curzerene (4.68; 4.78), and velleral (4.78; 3.91) respectively, found in both periods, at higher concentrations than 3.5%.

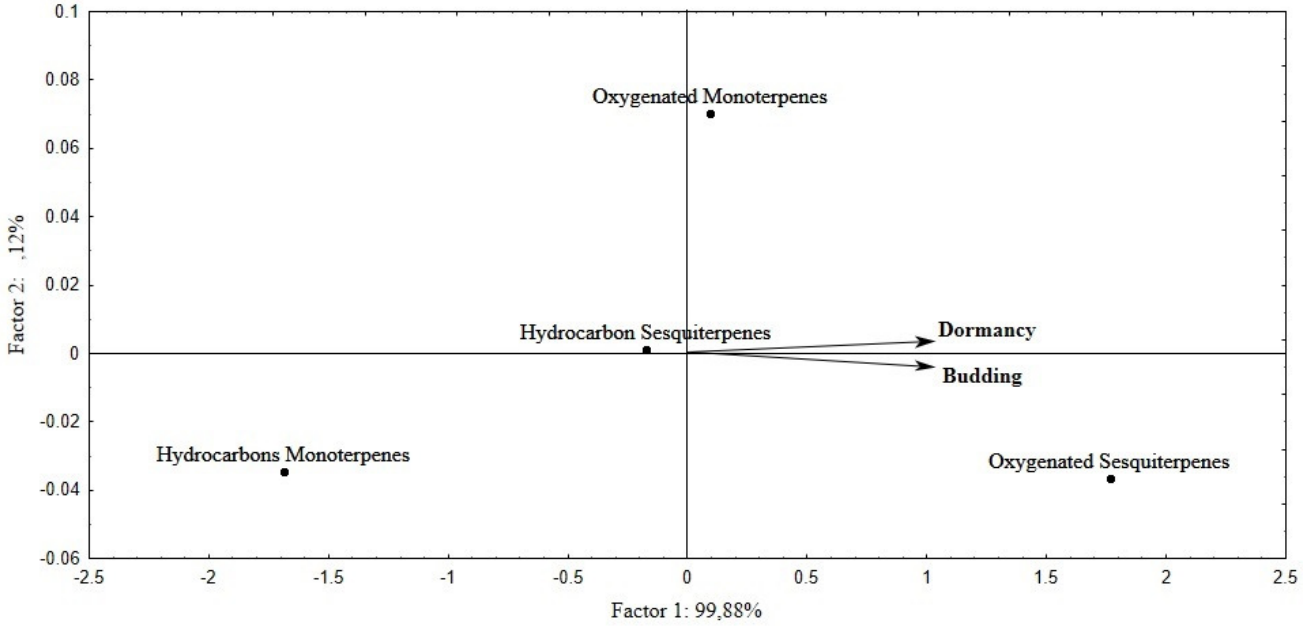


Figure 1. Biplot representing the projection of chemical classes of *Curcuma zedoaria* rhizome essential oil obtained during dormancy and budding periods

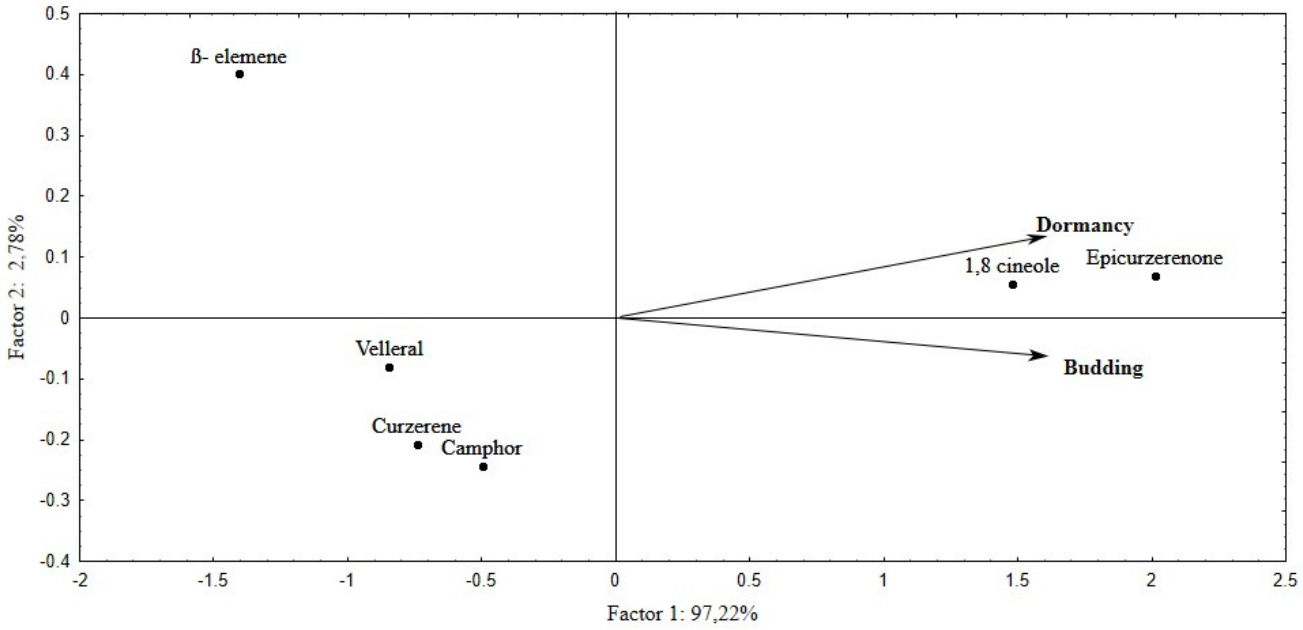


Figure 2. Biplot representing the projection of the major chemical compounds of *Curcuma zedoaria* rhizome essential oil obtained during dormancy and budding periods.

The differences observed in the chemical composition in both evaluated periods (Table 1 and Figures 1 and 2) occurred in function of *C. zedoaria* vegetative cycle which is well defined in this species. It starts with the growth of vegetative sprouts (buds) in the summer. Next, growth ceasing, bud formation, senescence and leaf abscission occur in the autumn, and bud dormancy in the winter (RINNE et al., 2010; DING; NILSSON, 2016). This cycle is dynamically modulated by intrinsic environmental and hormonal factors, and according to Horvath et al. (2003), the presence of the hormones ethylene and auxin and phytochrome protein act directly on the bud dormancy stage while gibberellic acid, abscisic acid and cytokine play antagonistic roles in dormancy, regulating specific components of the cellular cycle (ANDERSON et al., 2001). Regarding the influence of intrinsic and environmental factors (photoperiod and water deficit), the vegetative and reproductive meristems of several perennial plants in temperate climate remain in a latent state without growth during the cold period of the autumn and winter, within the buds, guaranteeing an optimal protection against low and dry temperatures (RIOS et al., 2014).

Regarding the major compounds found in this experiment, they differed from the ones found by Mau et al. (2003) who evaluated the chemical composition of EO of *C. zedoaria* rhizome from China, finding *epi*-curzerenone (24.08%), and curzerene (10.36%). In a zedoaria cultivation implemented in India, Singh et al. (2002) identified 1,8 cineole (18.50%) and *p*-cymene (18.42%). Yonzon et al. (2005) identified 1,8 cineole (15.8%) and *β*-eudesmol (10.61%). Purkayastha et al. (2006) identified curzerenone (22.3%), 1,8 cineole (15.9%) and germacrone (9,0%). Syamsir et al. (2017) also evaluated the chemical composition of zedoaria rhizomes from Malaysia and Indonesia and found camphor (17.6% and 19.7%), zerumbone (17.6% and 12.1%) and curzerenone (10.2% and 7.4%), respectively.

C. zedoária EO was evaluated against *Ae. aegypti* larvae and pupae by calculating the Lethal Concentrations (LCs) that are necessary to eliminate 50.0% (LC_{50}) and 99.9% ($LC_{99.9}$). The results are described in Table 2.

Table 2. Mean ± standard deviation and confidence interval of lethal concentrations (LC_{50} and $LC_{99.9}$) of essential oil of *Curcuma zedoaria* rhizome collected in the dormancy and budding periods against *Aedes aegypti* larvae and pupae by Probitos analysis.

		LC_{50} (mg mL^{-1}) (CI)	**$LC_{99.9}$ (mg mL^{-1}) (CI)**
Aedes aegypti **Larvae**	**D**	**0.0072^{a} ± 0.0005** (0.0069 – 0.0079)	**0.0111^{a} ± 0.0004** (0.0109 – 0.0116)
	B	**0.0158^{b} ±0.0020** (0.0140 – 0.0179)	**0.0821^{b} ± 0.0008** (0.0815 – 0.0830)
Aedes aegypti **Pupae**	**D**	**0.7800^{A} ± 0.0562** (0.6825 – 0.7800)	**1.3853^{A}±0.0375** (1.3636 – 1.4287)
	B	**0.8654^{A} ±0.1144** (0.7993 – 0.9975)	**2.6353^{B} ± 0.1344** (2.5577 – 2.7906)

LC_{50}: concentration of the oil that killed 50% of *Aedes aegypti* larvae and pupae; $LC_{99.9}$.: concentration of the oil that killed 99.9% of *Aedes aegypti* larvae and pupae; CI: confidence interval; different letters in the same column indicate significant differences between the treatments by Duncan's Test ($p \leq 0.05$). D: period in which the plant is in dormancy; B: period in which the plant is in budding.

The essential oil of *C. zedoaria* obtained in the periods of dormancy and budding killed *Ae. aegypti* larvae and pupae with $LC_{99.9}$ of (0.01 and 1.38 mg mL^{-1}) and (0.08 and 2.63 mg mL^{-1}), respectively (Table 2). The greatest activity was observed in dormancy EO, corroborating the results found in the chemical analysis in which it was possible to verify that the compounds epicurzerenone, 1,8 cineole and *β*-elemene are found in greater amounts. From these three compounds, 1,8 cineole and *β*-elemene can be responsible for the larvicidal action because, similarly in *C. zedoaria*, these compounds were the major ones in EOs of some species. One of the major compounds of EO from *Croton jacobinensis* (Euphorbiaceae) leaves and inflorescences was 1,8 cineole (16.90%), and when tested against *Ae. aegypti*, it presented LC_{50}= 0.0793 mg mL^{-1} for the leaves and LC_{50}= 0.0658 mg mL^{-1} for the inflorescences (PINTO et al., 2016). Regarding *β*-elemene, it is one of the major compounds of the EO of both species that were tested against *Ae.*

aegypti larvae. In *Toddalia asiatica* L (Rutaceae) EO, (10.67%) of this compound was found with LC_{99} =0.293 mg mL^{-1} (MAHESWARAN et al., 2016). In *Murraya exotica* L (Rutaceae) EO, (7.56%) was found with $LC_{99=}$ 0.152 mg mL^{-1} (KRISHNAMOORTHY et al., 2015). Epi-curzerenone presents high antimicrobial (PRAKASH et al., 2018) and antitumor activities (SEPTANINGSIH et al., 2018); however, there have been no reports showing its larvicidal potential.

We understand that because they are major compounds of this species, it would be important to isolate them to investigate if these compounds were responsible for the mortality of *Ae. aegypti* larvae and pupae.

Another point to be discussed is the differentiated action of EOs against larvae and pupae. The obtained results showed greater potential against larvae because the bio compounds acted on the interaction of the larval cell wall as well as in the ingestion and absorption by their gastrointestinal tract - (PROCÓPIO et al., 2015). However, in pupae, because they do not feed themselves, the biocompound penetration was more difficult (CHAUBEY 2012; PROCÓPIO et al., 2015; PIETA et al., 2017).

The aim of our study was to verify how *C. zedoaria* EO killed larvae and pupae. Therefore, the inhibitory potential of anticholinesterase enzyme, responsible for the transmission of nervous impulses, was determined by bioautographic method, and the results are shown in Table 3.

Table 3. Evaluation of the inhibitory potential of *Curcuma zedoaria* rhizome essential oil on anticholinesterase enzyme.

Concentration mg mL^{-1}	EO		
	Dormancy	Budding	Positive Control
10.00	+	+	+
5.00	+	+	+
2.50	+	+	+
1.25	+	+	+
0.625	+	+	+
0.312	+	+	+
0.156	+	+	+
0.078	+	-	+
0.039	+	-	+
0.019	-	-	+
0.009	-	-	+

EO: essential oil; positive control: organophosphate commercial solution; (+): inhibition of acetylcholinesterase enzyme; (-): absence of inhibition of acetylcholinesterase enzyme.

The dormancy EO presented greater inhibition on the acetylcholinesterase enzyme (0.039 mg mL^{-1}) when compared to the budding period (0.156 mg mL^{-1}), probably due to the presence of a greater amount of 1,8-cineol in dormancy EO as it presents larvicidal action already reported in the literature (LOBO et al., 2009; LIU et al., 2012; PINTO et al., 2016).

Comparing the LCs found for larvae (Table 2) and a smaller concentration that inhibited acetylcholinesterase enzyme (Table 3), it was evident that the *in vitro* test (bioautographic) showed lower effectiveness than the *in vivo* test against *Ae. aegypti* larvae. It can be suggested that this observed difference can be related to more than one action mechanism of the EO chemical compounds, besides the inhibition of the acetylcholinesterase enzyme. It is known that the conventional chemical larvicides, such as pyrethroids, act through different action mechanisms, which act on the neural sodium channels, interfering in their opening and closing, and extending the entry of Na^+ ions into the cell (SANTOS et al., 2007) while organophosphates inhibit acetylcholinesterase enzyme (BRAGA; VALLE, 2007). These results open new perspectives because our objective it to provide natural bioinsecticide that can act in synergism with conventional larvicides.

CONCLUSIONS

The essential oil (EO) of *Curcuma zedoaria* rhizome obtained in the dormancy and budding periods were evaluated against *Ae. aegypti* larvae and pupae.

The dormancy EO presented greater potential with $LC_{99.9}$ of (0.01 and 1.38 mg mL^{-1}), respectively.

The chemical analysis by GC/MS and grouping by PCA indicated the presence of epicurzerenone (18.20%); 1,8 cineol (15.76%) and *β*-elemene (4.43) with greater amount in dormancy period, indicating that these compounds can be responsible for the action against larvae and pupae.

The results were promising because they establish in which period of the vegetative cycle *C. zedoaria* EO presents greater bioinsecticide potential.

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RESUMO: Neste trabalho foi avaliado o potencial bioinseticida do óleo essencial (OE) extraído dos rizomas da espécie *Curcuma zedoaria* (Zingiberaceae), coletados no período de dormência (inverno) e brotação das gemas (verão). O OE foi obtido por hidrodestilação (2h) e identificado por CG/EM foi observado rendimento 0,61 ± 0,07 (%) no óleo da dormência, quando comparado no período de brotação 0,55 ± 0,08 (%). Os bioensaios sobre as larvas e pupas de *Aedes aegypti* foram realizados pelo teste de imersão em diferentes concentrações dos OEs, que variaram de 500,00 a 0,003 mg mL^{-1} (v/v). Os resultados sobre as larvas e pupas indicaram uma $CL_{99,9}$ de (0,01 e 1,38 mg mL^{-1}) para o OE da dormência, e (0,08 e 2,63 mg mL^{-1}) para o OE do período de brotação, respectivamente. Indicando maior atividade do OE da dormência. O mecanismo de ação dos OEs nos dois períodos foi determinado pelo método autobiográfico avaliando o potencial inibitório sobre a enzima acetilcolinesterase. Os resultados indicaram maior inibição da enzima do OE no período de dormência (0,039 mg mL^{-1}), quando comparado ao OE de brotação (0,156 mg mL^{-1}). A análise química destacou três compostos: epicurzerenone (18,20% e 12,10%) e 1,8 cineol (15,76% e 14,05%) e β- elemeno (4,43 e 0,01%) em maior quantidade no período de dormência quando comparado ao período de brotação, respectivamente. Esta diferença pode explicar a maior ação inseticida do OE de dormência sobre as larvas e pupas do *Ae. aegypti*. Os resultados são promissores, pois estabelece em qual período do ciclo vegetativo o OE da *C. zedoaria* apresenta maior potencial bioinseticida.

PALAVRA-CHAVE: *Aedes aegypti*. Anticolinesterase. 1,8-cineol. Epicurzerenone. *β*-Elemeno. Concentração letal (CL).

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