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FULL PAPER

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Composition and Larvicidal Activity of the Oil of *Dizygostemon riparius* (Plantaginaceae), a New Aromatic Species Occurring in Maranhão, Brazil

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Dizygostemon riparius (Plantaginaceae) is a new aromatic herbaceous species occurring in Maranhão State, Brazil. It is used as flavorings to remove domestic animal parasites and preventing mosquitoes. GC and GC/MS were used to analyze its essential oil, and a larvicidal bioassay was performed against *Aedes albopictus* larvae, a vector of arboviruses in Brazil. *endo*-Fenchyl acetate, *endo*-fenchol, (*E*)-caryophyllene, and caryophyllene oxide were the oil's primary constituents, totalizing 88.0%. The two morphotypes of *D. riparius*, with purple and white flowers, did not present a significant difference in the oils' composition. From a chemotaxonomic point of view, *D. riparius* oil showed some similarities with other oils of Plantaginaceae, suggesting (*E*)-caryophyllene and caryophyllene oxide as possible chemical markers. The oil larvicidal action displayed the lowest and highest mortality percentage at 50 mg/L (2.0%) and 600 mg/L (88.0%). The fenchyl acetate and fenchol standards showed a lower and higher mortality percentage at a concentration of 300 mg/L (42.0% and 26.0%) and 900 mg/L (96.0% and 98.0%), respectively. The present study results with the *D. riparius* oil point to a new bioproduct with significant larvicidal activity.

Keywords: Plantaginaceae, new aromatic species, essential oil, *endo*-fenchyl acetate, *endo*-fenchol, larvicidal activity.

Introduction

In the last 20 years, systematic molecular specialists have reshaped the understanding of angiosperm evolution, culminating in new classifications. Botanists are faced now with families whose circumscriptions have sharply changed. More recent phylogeny works revealed that Scrophulariaceae, as traditionally recognized, is not monophyletic and now has a more restricted concept. Before in Scrophulariaceae, now the genus *Dizygostemon* (Benth.) Radlk. ex Wettst. belongs to Gratioleae, the mainly tropical tribe of Plantaginaceae, comprising ca. 25 genera and over 300 species.[1,2]

Dizygostemon riparius Scatigna & Colletta (Plantaginaceae) is an aromatic sub-shrub, up to 50 cm tall, with occurrence in the State of Maranhão, Brazil, and had its botanical description recently reported.[3] It is known as 'melosa' by the local population of the municipality of São Benedito do Rio Preto, MA, Brazil, due to its viscous characteristic. Its leaves and thin stems have a refreshing and pleasant aroma, being used as flavorings, cleaning parasites of domestic animals, and preventing mosquitoes. In addition to *D. riparius*, there are only one other species of *Dizygostemon*. It is *D. floribundum* (Benth.) Radlk. ex Wettst. (syn. *D. angustifolium* Giul.), occurring in areas of Caatinga

of the states of Bahia, Pernambuco, Ceará, and Piauí, in Northeastern Brazil.[3]

The essential oils act as physical and chemical barriers and inhibition agents against microorganisms and insects which threat humans, animals, and plants.[4,5] Thus, the intensity of scientific research focused on essential oils, with suitable active substances for new botanical larvicides, has been growing over the past twenty years. A good example is Pavela (2015)[6] work describing essential oils as potential larvicides, based on their chemical composition and biological efficacy. The selected essential oils were required to meet the following conditions: a chemical composition had to be known and the $LC_{50} \leq$ 100 ppm.[6] Some essential oils with high potential as a larvicide against *Aedes aegypti* (Linnaeus, 1762) and *A. albopictus* (Skuse, 1894) (Diptera: Culicidae) are: *Lippia sidoides* Cham., with thymol and α-phellandrene as the primary constituents.[7] *Ocimum basilicum* L., with linalool and methyl eugenol as main components;[8] and *Stemodia maritima* L., with (*E*)-caryophyllene and caryophyllene oxide as significant compounds.[9] In the three examples above, with essential oils that have bioactive compounds and significant larvicidal activity, it can be seen that different pathways biosynthesize their main constituents: monoterpenoids, sesquiterpenoids, and phenylpropanoids, respectively.

This work aimed to obtain and analyze, for the first time, the chemical composition of essential oil of the *D. riparius* morphotypes and to evaluate its larvicidal activity, given the widespread use of the plant in pest control. The larvicidal oil test was conducted with larvae of the mosquito *Aedes albopictus*, a vector of arboviruses in rural and semi-urban areas spread worldwide and, particularly, in Brazil.

Results and Discussion

Oil Composition Analysis

Oil of *D. riparius* showed an average yield of 1.3% (v/w). The constituents of the oils of the purple and white flower morphotypes are displayed in *Table 1*. They were identified and quantified by GC/MS and GC-FID, respectively. Thirty-four compounds were identified, representing 97.3% of total oils composition, excluding a small percentual of unidentified diterpenes (in medium 1.30%; MW from 282 to 286). The oxygenated monoterpenes *endo*-fenchyl acetate (42.8% to 48.0%) and *endo*-fenchol (33.3% to 35.0%) were the primary constituents. They were followed by the sesquiterpene hydrocarbon, (*E*)-caryophyllene (4.2% to 6.8%), and the oxygenated sesquiterpene, caryophyllene oxide (2.3% to 3.3%).

A small increase in the percentage of the main oxygenated monoterpenes in air-dried plant oils was observed in comparison to oils from plants submitted to freeze-drying. Likewise, a higher percentual of the main sesquiterpene hydrocarbons in oils from lyophilized plants was observed when compared to oils from air-dried plants, suggesting that in the lyophilization process there seems to be more noticeable volatilization of the oil constituents. No significant difference was observed between the oils of the white (BN17 and BL17) and purple (RN17 and RL17) morphotypes, appearing to be only a small genotypic variation.

In the analyzed oils of *D. riparius*, the oxygenated monoterpenes *endo*-fenchyl acetate and *endo*-fenchol were the main constituents, with an average percentage of 80%, followed by about 8.0% of (*E*)-caryophyllene plus caryophyllene oxide. The chemical structures of the main constituents of *D. riparius* oil are displayed in *Figure 1*. The oil presented a refreshing, pleasant, lemon, and camphor aroma due to both constituents' significant presence. No other essential oil with a high *endo*-fenchyl acetate and *endo*-fenchol combined content has been previously found in the literature. Differently, essential oils from rhizomes of some *Alpinia* (Zingiberaceae) species have shown a significant percentage of *endo*-fenchyl acetate, as in the oil of *A. galanga* (L.) Willd., where the content of *endo*-fenchyl acetate was 54%, and in the oils of *A. calcarata* Rosc. and *A. speciosa* K. Schum. [syn. *A. zerumbet* (Pers.) Burtt & Smith], where this value was 40% for both species.[10–13] The same was observed to *endo*-fenchol, where the leaf oils of *Tarchonanthus camphoratus* L. (Asteraceae) and *Ocimum forskolei* Benth.

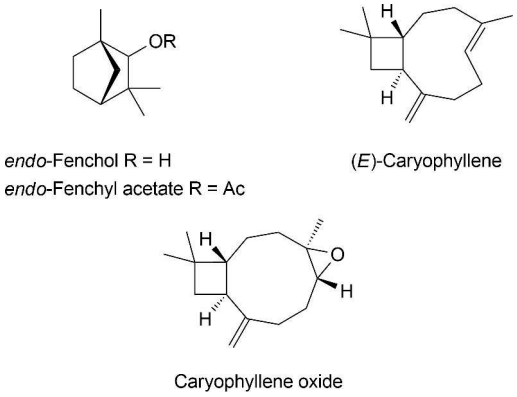

Figure 1. Structures of primary constituents of *D. riparius* essential oil.

Table 1. Yields and constituents of the essential oils of *Dizygostemon riparius*.[a]

Oil yields (%) Oil constituents (%)	$RI_{Calc.}$	$RI_{Lit.}$	1.2 BN17	1.3 BL17	1.4 RN17	1.1 RL17
α-Fenchene	939	945	0.2	0.2	0.7	0.5
α-Terpinene	1005	1014			0.1	0.1
p-Cymene	1013	1020	0.3	0.3	0.5	0.6
Limonene	1018	1024	0.3	0.4	0.6	
γ-Terpinene	1049	1054			0.2	
Fenchone	1082	1083	0.6	0.8	0.8	0.5
endo-Fenchol (α-Fenchol)	1118	1114	35.0	34.1	33.5	33.3
exo-Fenchol (β-Fenchol)	1131	1118		0.1		
neo-Isopulegol	1150	1144		0.1		
neoiso-Isopulegol	1175	1167	0.4	0.4	0.5	0.7
p-Cymen-8-ol	1186	1179	0.1	0.2	0.1	
α-Terpineol	1191	1186	0.4	0.2	0.1	
endo-Fenchyl acetate	1222	1218	45.8	42.8	48.0	44.4
Carvacrol	1290	1298	0.1		0.1	
neoiso-Isopulegyl acetate	1305	1312	0.2	0.3	0.2	0.3
neoiso-Dihydrocarveol acetate	1351	1356	0.7	0.9	1.0	0.8
trans-Myrtanol acetate	1392	1385	0.1	0.1	0.1	0.2
(*E*)-Caryophyllene	1419	1417	4.2	6.1	4.3	6.8
trans-Nerone	1444	1438	1.1	1.6	1.2	1.5
8α-Humulene	1452	1452	0.6	0.7	0.4	0.7
Italicene epoxide	1551	1547	0.2	0.1	0.1	
epi-Longipinanol	1558	1562			0.1	
(*E*)-Nerolidol	1561	1561	0.1	0.1	0.1	
Spathulenol	1575	1577	0.6	0.8	0.3	0.5
Caryophyllene oxide	1584	1582	2.3	3.3	2.6	3.0
Khusimone	1599	1604	0.3	0.2	0.2	
Humulene epoxide II	1608	1608	0.2	0.1	0.2	
Caryophylla-4(12),8(13)-dien-5α-ol	1634	1639	0.5	0.7	0.3	0.3
Caryophylla-4(12),8(13)-dien-5β-ol	1637	1639	1.8	1.9	1.3	1.5
Pogostol	1652	1651	0.6	0.3	0.2	0.2
allo-Himachalol	1656	1661	0.4	0.2	0.2	
14-Hydroxy-9-*epi*-(*E*)-caryophyllene	1669	1668	0.7	0.5	0.3	0.2
Abietatriene	2054	2055	0.2	0.1	0.1	
Diterpenes not identified (MW 282–286)	2241–2278		1.4	1.4	1.3	1.4
Total (%)			99.4	99.0	99.7	97.5

[a] BN17 and RN17 = air-dried white and purple flower morphotypes; BL17 and RL17 = lyophilized white and purple flower morphotypes; $RI_{Calc.}$ = calculated retention time (on Rxi-5 ms column); RI_{Lit} = literature retention time.

(Lamiaceae) have shown high content of *endo*-fenchol, with 21 % and 31 %, respectively.[14,15]

In Brazil, the only genera of Plantaginaceae with native representatives are *Achetaria* Cham. & Schltdl., *Dizygostemon*, *Stemodia* L., and *Tetraulacium* Turcz. According to Sousa and Giullietti (2009),[2] at the general level, there is a close relationship between *Achetaria*, *Dizygostemon*, and *Tetraulacium*. Moreover, in many Brazilian herbaria, specimens of *Achetaria*, *Dizygostemon*, and *Tetraulacium* have been identified as *Stemodia*, the largest genus of the group, due to their resemblances and suggesting which there is a close phylogenetic relationship between them (Souza and Giulietti 2009; BFG 2015, 2018).[2,16,17] Also, according to Ronse (2001),[18] the Brazilian *Otacanthus* Lindl. genus could be considered the closest to the *Achetaria* genus due to the common characteristics in the stamens and anthers.

As it is a recently described aromatic species, *Dizygostemon riparius* lacks scientific information regarding its chemical composition and biological properties. Only some works of species belonging to other genera of the family can be found in the literature. The essential oil of leaves and thin stems of *Stemodia maritima* L., collected in the State of Ceará, Brazil, showed (*E*)-caryophyllene, caryophyllene oxide, and 14-hydroxy-9-epi-(*E*)-caryophyllene as primary constituents.[9] In *D. riparius* oil these sesquiterpenes

exist as secondary compounds, in addition to a significant percentage of the oxygenated monoterpenes *endo*-fenchyl acetate and *endo*-fenchol (see *Table 1*). Also, from the aqueous and ethanol extracts of *S. maritima* were isolated stemodin, D-mannitol, betulinic acid, two derivatives of β-sitosterol and stigmasterol, and the description of a new diterpene compound.[19] In the oils of *Stemodia trifoliata* (Link) Rchb. and *Stemodia foliosa* L., sampled at Guarapiranga, the state of Ceará, and São Lourenço da Mata, State of Pernambuco, Brazil, respectively, have predominated the diterpenes 6α-hydroxymanoyl oxide, 6α-acetoxymanoyl oxide, and 6α-malonyloxybutyl ester manoyl oxide, followed by (*E*)-caryophyllene and caryophyllene oxide,[20,21] the same sesquiterpenes found in *D. riparius* oil. Previously, it was reported that the genera *Otacanthus* and *Achetaria* would be very close,[22] at the same time that *Achetaria* would have presented a phylogenetic relationship with *Dizygostemon*.[2] In the volatile fraction of *Otacanthus coeruleus* Lindl.[23] and in the essential oil of *O. azureus* (Lindl.) Ronse [syn. *Achetaria azurea* (Lindl.) V.C. Souza],[24] the sesquiterpenes β-copaen-4-α-ol, α-humulene, and α-copaene, and the monoterpenes myrtenal, *trans*-pinocarveol, and pinocarvone were their primary constituents. Therefore, presenting a very different chemical profile from that found in *D. riparius* oil.

Larvicidal Bioassay

Aedes albopictus larvae used in the bioassays resulted from the insect eggs collected on Paço do Lumiar city, Maranhão State, Brazil, where many cases of infection by Dengue and Chikungunya viruses have been confirmed. The results for *A. albopictus* larvae population mortality to the emulsified formulations of the *D. riparius* oil and the fenchyl acetate and fenchol standards, after 24 h of exposure, are shown in *Table 2*. It should be considered that there are no previous studies for the biological activity of *D. riparius* oil, and analogously, no reports were found with larvicidal tests to fenchyl acetate and fenchol, its primary constituents.

The oil (EO) larvicidal action was intensified according to the increase in concentration, displaying the lowest mortality percentage at 50 mg/L (2.0%) and the highest at 600 mg/L (88.0%). The fenchyl acetate (FA) and fenchol (F) standards showed a lower mortality percentage, 42.0%, and 26.0%, respectively, at a concentration of 300 mg/L. These standards also presented the higher dead larvae percentage, 96.0% to fenchyl acetate and 98.0% to fenchol, at the concentration of 900 mg/L. The accumulation of dead larvae was not linear to the increased concentration, which could evidence the larvae's possible genetic variability in the treatments applied.

The lethal concentrations (LC_{50} and LC_{90}) of the oil and standards were determined by probabilistic analysis (Probit), as depicted in *Figure 2*. The curves accumulated mortality values as a function of the log concentration, exhibiting low mortality percentage at concentrations below 300 mg/L (2.48) and a high mortality rate at concentrations above 400 mg/L (2.60). According to the LC_{50} values, the lethality efficiency increases from the fenchol to the essential oil (F < FA < EO). On the other hand, analyzing the LC_{90} values indicates that the lethality increased from the essential oil to fenchol (EO < FA < F).

For a better evaluation of lethality, in *Table 3*, it is seen that the Probit slope has a more significant correlation with the CL_{90} values. The angular coef-

Table 2. Larvicidal activity of the oil of *D. riparius* and the fenchyl acetate and fenchol standards against *Aedes albopictus* larvae.[a]

Concentration (mg/L)	Log [ ]	Accumulation of dead			Mortality (%)		
		EO	FA	F	EO	FA	F
900	2.95		48.0 ± 0.55	49.0 ± 0.45	–	96.0	98.0
800	2.90		47.0 ± 0.55	48.0 ± 0.55	–	94.0	96.0
700	2.84		41.0 ± 0.84	47.0 ± 0.55	–	82.0	94.0
600	2.78	44.0 ± 1.30	42.0 ± 0.55	46.0 ± 0.45	88.0	84.0	92.0
500	2.70	38.0 ± 1.82	37.0 ± 0.89	42.0 ± 0.89	76.0	74.0	84.0
400	2.60	31.0 ± 1.48	38.0 ± 0.55	44.0 ± 1.64	62.0	76.0	88.0
300	2.48	20.0 ± 2.12	21.0 ± 0.45	13.0 ± 0.55	40.0	42.0	26.0
200	2.30	10.0 ± 1.58			20.0		
100	2.00	10.0 ± 0.71			20.0		
50	1.70	1.00 ± 0.45			2.0		

[a] Log [ ] = Logarithmic concentration; Accumulation dead = Number of dead larvae; FA = Fenchyl acetate; F = Fenchol.

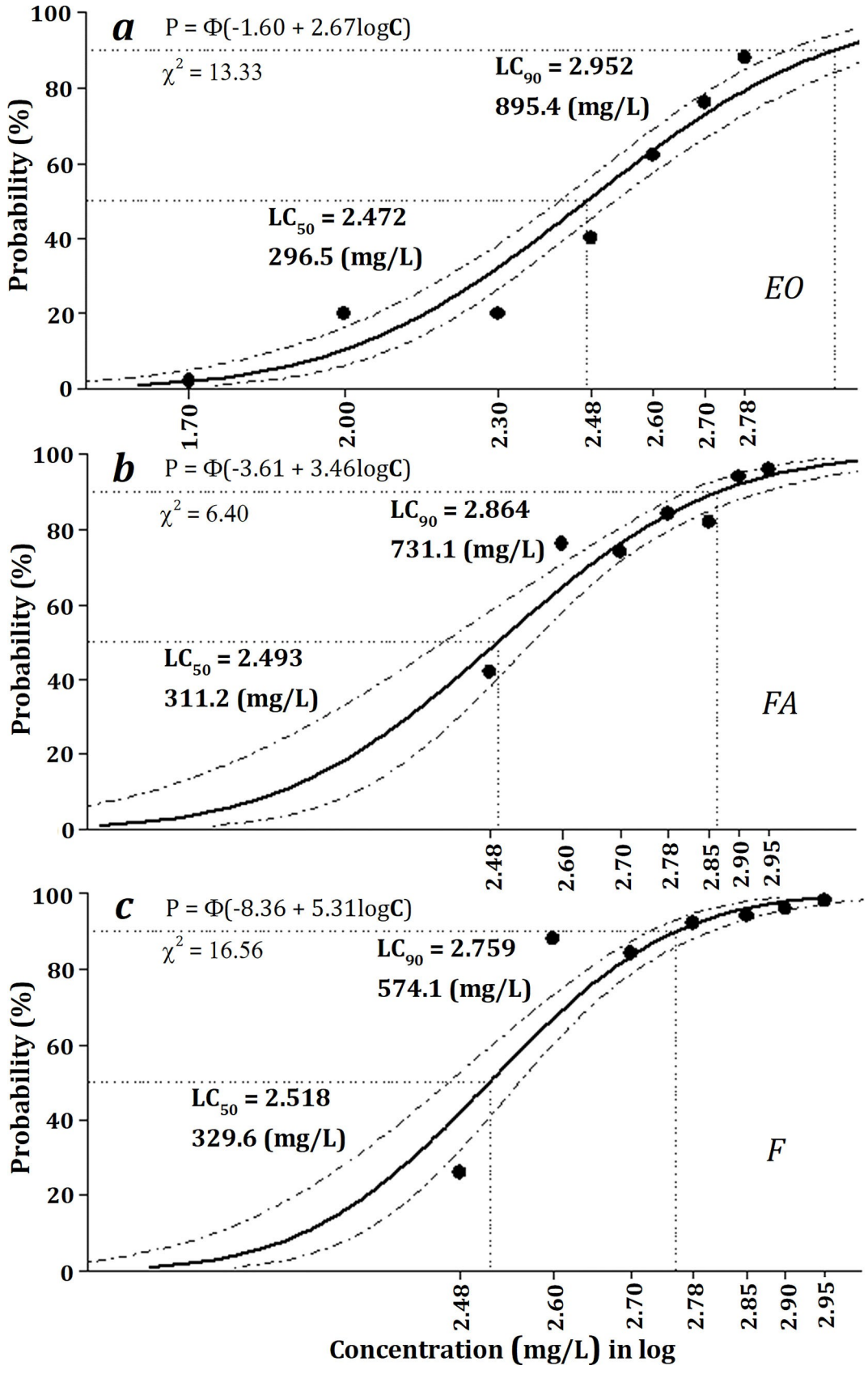

Figure 2. Larvicidal activity. Concentration-mortality curves (LC_{50} and LC_{90}) for the treatments with *D. riparius* oil (EO) and the fenchyl acetate (FA) and fenchol (F) standards.

Table 3. The lethal concentration of the larvicidal agents based on Probit analysis: essential oil of *D. riparius*, fenchyl acetate, and fenchol.[a]

Larvicidal agents	Probit analysis			
	LC_{50} (mg/L)	LC_{90} (mg/L)	Slope ± SE	χ^2
Oil	296.5	895.4	2.67 ± 0.46	13.33
Fenchyl acetate	311.2	731.1	3.46 ± 0.58	6.40
Fenchol	329.6	574.1	5.31 ± 1.17	16.56

[a] Slope = Probit slop; SE = Standard error; χ^2 = Chi-square.

ficient value of fenchol (5.31 ± 1.17) is about twice times greater than the essential oil value (2.67 ± 0.46) and about once and a half the fenchyl acetate (3.46 ± 0.58) value. Based on this, it is assumed that fenchol is the most lethal larvicidal agent in the *D. riparius* essential oil matrix. However, when comparing the CL_{50} and CL_{90} values of fenchol with the essential oil matrix (see *Table 3*), an antagonistic effect is observed. The inversion in the order of lethality indicates that the fenchol larvicidal activity displays a reduced value when associated with the oil matrix. The same action is observed for fenchyl acetate, showing an antagonistic effect on the two significant constituents' oil matrix.

This work presents the first result for the larvicidal potential of a Plantaginaceae species against larval populations of *A. albopictus*, an invasive mosquito, and considered potential transmitter of endemic diseases in Brazil. Some authors point to the larvicidal efficiency of essential oils based on the 50% lethal concentration (LC_{50}), establishing that LC_{50} values less than 50 mg/L are very active, LC_{50} values between 50 and 100 mg/L are active, LC_{50} values between 100 and 750 mg/L are effective, and LC_{50} values above 750 mg/L are inactive.[16,25] However, it is reiterated that the LC_{90} value must be considered a relevant criterion to determine larvicidal efficiency, since it represents a better approximation of the maximum mortality percentage, as seen in the probabilistic analysis of the present bioassay.

The chemical structure of essential oil constituents (as mono- and sesquiterpenes) and the insecticidal/larvicidal activity is related to their lipophilicity. The higher the lipophilicity, the better the penetration of the components in the integument of insects/larvae. It is assumed that the *D. riparius* volatile constituents must have high permeability in the larvae tissue, because they are low polarity compounds and with high hydrophobic character. Studies involving the action of essential oils constituents on insects/larvae suggest other mechanisms besides effect on the octopaminergic nervous system, such as inhibition of acetylcholinesterase (AchE) and gamma-butyric acid (GABA) receptors.[26,27]

The World Health Organization (WHO) does not establish lethal concentration parameters to characterize natural products' larvicidal potential, as the essential oils. It recommends using standard dosage adopted for synthetic products. In Brazil, the synthetic larvicides are based on their effectivity against *Aedes aegypti*, the dengue and chikungunya arboviruses' primary vector. Currently, the pesticide pyriproxyfen has been the main larvicidal agent recommended by health agencies in Brazil, which acts to inhibit the development of adult insects' characteristics, exhibiting teratogenic effects in the larval phase of arthropods. A recent study by Carvalho and co-workers (2020)[28] reaffirmed this proposition, with the discovery that mortality in the field of *Aedes aegypti* larvae varies from 06. to 2.0%, while mortality from pupae is 88 to 99%, in the dose of 30 ng/mL.

It should be noted that synthetic larvicidal products used in the continuous fight against mosquitoes have developed resistance in the *A. aegypti* and *A. albopictus* populations. On the other hand, the results of the present study with the *D. riparius* oil point to a bioproduct with larvicidal activity, based on the synergistic action of the multiple mechanisms exhibited by the volatile constituents present on its oil matrix, which can inhibit the process of natural resistance demonstrated by *Aedes* species. Further tests will be necessary so that the essential oil formulation can be used as a bioproduct for on-site application. However, in principle, it could be reflected in the application of mosquito breeding sites, in which the larvae have appropriate conditions for their development, particularly in the area where the plant occurs.

Conclusions

For the first time, the present work describes the chemical composition of the morphotype oils of *Dizygostemon riparius*, a new aromatic species from Maranhão, Brazil, with a predominance of *endo*-fenchyl acetate and *endo*-fenchol, as well as presenting the results of its significant larvicidal action.

Experimental Section

Plant Material and Collection Data

The botanical material of *Dizygostemon riparius* was sampled in the Rio Preto banks (geographic coordinates: 03°19′27.9″ S; 43°31′02.6″ W), Municipality of São Benedito do Rio Preto, State of Maranhão, Brazil, in the period from January to December 2017. It is an herbaceous plant about 50 cm long, recently described by the botanists André Vito Scatigna and Gabriel Dalla Colletta, from the Departamento de Biologia Vegetal at Universidade Estadual de Campinas, Campinas, Brazil, deposited there under the accession number 202426.

Essential oil Distillation

The plant (leaves, thin stems, and flowers, 90 g) was subjected to hydrodistillation using a Clevenger type glass apparatus (3 h). Before, separately, the plant samples were dried in air at room temperature for five days and lyophilized for two days, then, subjected to grinding with a cyclone mill. After the extraction, the oils were dried over anhydrous sodium sulfate, and their yields calculated based the plant dry weight (v/w). The moisture content of the samples was calculated using an Infrared Moisture Balance for water loss measurement. The oil extraction was performed with the purple and white flowers plant morphotypes, separately, and duplicate procedures. Four samples of the plants were processed: BN17, the white flower morphotype, air-dried at room temperature; BL17, the white flower morphotype, dried by lyophilization; RN17, the purple flower morphotype, air-dried at room temperature; and RL17, the purple flower morphotype, dried by lyophilization.

Oil Composition Analysis

Analysis of the oils was performed on a GCMS-QP2010 Ultra system (Shimadzu Corporation, Japan), equipped with an AOC-20i auto-injector and the GCMS-Solution software containing the NIST and FFNSC 2 libraries.[29,30] A Rxi-5 ms (30 m × 0.25 mm; 0.25 μm film thickness) silica capillary column (Restek Corporation, Bellefonte, PA, USA) was used. The conditions of analysis were: injector temperature of 250 °C; Oven temperature programming of 60–240 °C (3 °C min^{-1}); Helium as the carrier gas, adjusted to a linear velocity of 32 cm s^{-1} (1.0 mL/min); split mode injection for 1.0 μL of the sample (oil 5.0 μL: hexane 500 μL); split ratio 1:20; ionization by electronic impact at 70 eV; ionization source and transfer line temperatures of 200 and 250 °C, respectively. The mass spectra were obtained by automatic scanning every 0.3 s, with mass fragments in 35–400 *m/z*. The retention index was calculated for all volatile components using a homologous series of C_8-C_{40} *n*-alkanes (Sigma–Aldrich, USA), according to Van Den Dool and Kratz's linear equation.[31] The quantitative data regarding the volatile constituents were obtained by peak-area normalization using a GC 2010 Series, coupled with FID Detector, which operated under similar conditions of the GC/MS system. The components were identified by comparing their retention indices and mass spectra (molecular mass and fragmentation pattern) with those existing in the GC/MS-Solution system libraries and the Adams library.[32]

Larvicidal Bioassay

It was performed according to methodology recommended by the World Health Organization[33] with adjustments, using an *Aedes albopictus* larvae field population, whose eggs were collected from traps placed in the Parque Jair, a rural area of the Paço do Lumiar city, State of Maranhão, Brazil (02°30′45" S/ 44°10′46.2" W). The eggs were transferred to polypropylene containers with mineral water at 28 °C, and the larvae growth was monitored until the 4th larval stage. The essential oil (EO) and fenchyl acetate (FA) and fenchol (F) formulations were prepared from a stock solution of 1000 mg/L each. The standards fenchyl acetate (96%) and fenchol (99%) were purchased from Sigma-Aldrich. Two negative control groups were tested, the first containing Tween 80 (0.5%) and the second containing only deionized water, according to the methodology used by Silva and colleagues (2008)[34] with adjustments. The larvicidal tests with the oil and standards included seven concentrations each. For the oil, concentrations were 50 and 100 to 600 mg/L, with 100 mg/L intervals. For each standard, concentrations ranged from 300 to 900 mg/L, with 100 mg/L intervals. The sampling size consisted of 1150 larvae, distributed in three treatments, and two negative control groups and was performed five times with ten larvae in each replicate. Larvae identification, after the 24 h test period, was made using an optical microscope, based on visualization of the scales of the 8th larval segment and their comparison with larval identification keys for *Aedes* species commonly found in Brazil.[35]

Statistical Analysis

Experimental data are presented through mean and standard deviation. The confidence limit was set at 95% ($p < 0.05$). The lethal concentrations (LC_{50} and LC_{90}) and their respective confidence intervals (Chi-square) were calculated through Probit analysis, using the R software version 3.5.3 (R Core Team, 2019).[36]

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Author Contribution Statement

C. M. Brandão – carrying out the experiments, data analysis, writing, and reviewing. K. S. B. Cavalcante, R. M. Teles, and J. G. S. Maia – data analysis, writing, and reviewing. G. E. C. Marques – data analysis and reviewing. O. S. Monteiro – reviewing. E. H. A. Andrade – data analysis.

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