Revista Brasileira de Farmacognosia 28 (2018) 135–144

Brazilian Journal of Pharmacognosy
REVISTA BRASILEIRA DE FARMACOGNOSIA
www.elsevier.com/locate/bjp

Original Article

Influence of abiotic environmental factors on the main constituents of the volatile oils of *Tithonia diversifolia*

Bruno Leite Sampaio, Fernando Batista Da Costa*

AsterBioChem Research Team, Laboratorio de Farmacognosia, Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, SP, Brazil

ARTICLE INFO

Article history:
Received 29 November 2017
Accepted 27 February 2018
Available online 21 March 2018

Keywords:
Volatile oils
Chemotypes
Terpenes
Environmental influence

ABSTRACT

Tithonia diversifolia (Hemsl.) A. Gray, Asteraceae, commonly known as Mexican sunflower, is a wide distributed invasive species encountered around the world. We proposed herein to establish the relationship between different abiotic environmental factors and the variation in the production of volatile compounds in *T. diversifolia*, during a period of one year. Samples of leaf and inflorescence volatile oils obtained from individuals located at two different regions of Brazil were analyzed by GC–MS and the data were submitted to chemometric analysis. Based on the main constituents, the analysis allowed us to classify the volatile oils into two chemotypes, according to their geographical origin. The influence of soil nutrients, mainly Ca and P, was also observed in the composition of the volatile oils. Climate also seems to affect the constituents of the volatile oils, mainly the contents of leaf sesquiterpenes of individuals growing in areas with higher average temperatures and solar radiation levels. We can therefore highlight that the appropriate multivariate statistical analysis allowed us to propose for the first time the existence of chemotypes for the volatile oils of *T. diversifolia*, as well as reporting the main abiotic environmental factors related to the accumulation of the discriminant compounds in these oils.

Introduction

The production and accumulation of metabolites in individuals of a same species growing at different regions may be influenced by environmental factors, so that such substances appear to play a role as a chemical interface between the plant and the surrounding environment (Ramakrishna and Ravishankar, 2011; Gutbrodt et al., 2012; Pavarini et al., 2012). This variation in composition and production of certain classes of secondary metabolites according to different environmental conditions can be used for characterization of different groups or populations of one species. In this sense, one or more metabolites may be pointed as chemical markers for specimens of a specified geographical region, season of the year and/or phenological phase, based on qualitative and quantitative analysis of their chemical constituents (Silva et al., 2006; Telascrea et al., 2007; Nehme et al., 2008; Jones et al., 2013; Vilela et al., 2013).

In plants that produce volatile oils (a complex mixture of volatile compounds, usually derived from the biosynthetic pathways of terpenoids or phenylpropanoids) (Figueiredo et al., 2008), the interaction with the environment is often related to variations in the production of these oils (composition and concentration of its constituents) (Telascrea et al., 2007; Lukas et al., 2009; Rahimmalek et al., 2009; Stashenko et al., 2010; Vilela et al., 2013).

Tithonia diversifolia (Hemsl.) A. Gray, commonly known as Mexican sunflower, is a plant species that produces volatile oil largely spread around the world. This species is a perennial herb from the family Asteraceae, tribe Heliantheae, native from both Mexico and Central America, and it can be encountered mainly in the both tropical and sub-tropical areas of Americas, Africa and Asia (Moronkola et al., 2007; Sánchez-Mendoza et al., 2011; Chagas-Paula et al., 2012). *T. diversifolia* is described as an invasive weed in different ecosystems, mainly in Africa and China (Ayeni et al., 1997; Sun et al., 2007; Muoghalu, 2008), which is related to problems of ecological imbalance in areas infested with this plant because it is able to adapt to different climate and soil conditions (Muoghalu and Chuba, 2005; Muoghalu, 2008).

The chemical constituents of *T. diversifolia* are well known and many of its secondary metabolites are described in literature. Most of the compounds isolated from *T. diversifolia* extracts belong to the classes of the sesquiterpene lactones, flavonoids and caffeoylquinic acid derivatives (Chagas-Paula et al., 2012; Zhao et al., 2012). A recent publication by our research group describes the relationship between the LC–MS-based metabolic profile of *T. diversifolia* and some abiotic environmental factors (soil nutrients and climate

---

* Corresponding author.
E-mail: febcosta@fcfrp.usp.br (F.B. Da Costa).

https://doi.org/10.1016/j.bjp.2018.02.005

factors) for individuals grown in two distinct regions of Brazil during a period of 24 months. The results demonstrate a clear and direct influence of the environmental conditions on the production and accumulation of some classes of non-volatile secondary metabolites, especially terpenes and phenolics, in four different plant tissues (leaves, stems, roots and inflorescences) (Sampaio et al., 2016).

The composition of the volatile oils from the leaves and inflorescences of specimens of *T. diversifolia* collected in Nigeria, Cameroon, South Africa and Vietnam are described in literature. The oils obtained from Nigerian samples are characterized by the major presence of hydrocarbon mono and sesquiterpenes, especially α-pinene and β-caryophyllene (Moronkola et al., 2007), while the oils from the Cameroon samples are characterized by the major components *cis*-β-ocimene (leaf) (Lamaty et al., 1991) and α-pinene (inflorescence) (Menut et al., 1992). The monoterpene α-pinene is the major constituent of the volatile oils of leaves and inflorescences obtained from South Africa (Lawal et al., 2012), as well as of leaf samples from Vietnam (Dai et al., 2015).

A recently published review article describes some differences for the chemical constituents of volatile oil samples of *T. diversifolia* originated from different regions of Africa, leading the authors to infer that the observed variability for the volatile oil composition can be related to environmental and climatic factors as well as geographical distribution (Ajao and Moteetee, 2017). Nevertheless, to the best of our knowledge, so far there is not any report describing the effect of the seasonality, phenological phase or the abiotic environmental factors on the composition of the volatile oils of *T. diversifolia* from different regions.

Considering the wide distribution and adaptive capacity of the Mexican sunflower as an invasive species and the lack of studies covering this subject, as well as the medicinal properties of this plant (Ajao and Moteetee, 2017; Chagas-Paula et al., 2012), we proposed herein to carry out a comparative study with samples of volatile oils from leaves and flowers of *T. diversifolia* obtained from specimens located in two different regions of Brazil and collected in different seasons throughout a year. Our approach involves comparing the data obtained by chemical analysis of these oils with the environmental data (climate and soil) from both regions, with the aiming to observe the existence or the absence of a seasonal pattern and also a relationship with the environmental factors in the production of volatile oils in *T. diversifolia*.

Materials and methods

Plant material

The volatile oils from *Tithonia diversifolia* (Hemsl.) A. Gray, Asteraceae, used to perform this study were obtained from six adult specimens of the plant, located in two different states of Brazil, in order to observe the effect of the different environments on the volatile oil production in *T. diversifolia*. Three adult specimens from one cultivated population of the species located at Campus of Ribeirão Preto of the University of São Paulo (USP-RP), municipality of Ribeirão Preto, state of São Paulo, and other three specimens from cultivation area located at the Santo Antonio Farm, municipality of Pires do Rio, state of Goiás, were selected for the study.

Leaves and inflorescences were collected by BL Sampaio at dawn, before the sunrise, from the six specimens located at the states of São Paulo and Goiás (three in each state), in the months of January, May, June, July, August and December of the year of 2013, thus contemplating a complete reproductive cycle of the plant. Voucher specimens for each sampled population were deposited and identified at Herbarium SPF, Institute of Biosciences, University of São Paulo, under the responsibility of the curation of Dr. Renato de Mello Silva. Voucher numbers for each specimen were assigned as Sampaio #01 (samples from Pires do Rio-GO) and Sampaio #02 (samples from Ribeirão Preto-SP).

The samples from Goiás, immediately after the harvesting, were frozen in dry ice in order to avoid any alteration in composition of the volatile oils. The samples were then transported to the Laboratory of Pharmacognosy of the School of Pharmaceutical Sciences of Ribeirão Preto of the University of São Paulo (FCFRP-USP). Samples from São Paulo were collected and immediately taken to the Laboratory of Pharmacognosy (FCFRP-USP), where the extraction of the volatile oils of all samples was performed.

Extraction of volatile oils

The volatile oils of *T. diversifolia* were obtained from fresh leaves and inflorescences by the process of hydrodistillation performed in a Clavenger apparatus as described by Brazilian Pharmacopoeia modified (Anvisa, 2010), and after the extraction, the oils were storage in microtubes and kept in a freezer at a temperature of −20 °C.

Analysis of the volatile oils by GC–MS

The volatiles oils from *T. diversifolia* were analyzed by gas chromatography coupled to mass spectrometry in an instrument model Gas Chromatograph Mass Spectrometer – Shimadzu® – QP 2010, using a DB-5MS column (30 m × 0.25 mm × 0.25 μm) Agilent®, employing the following method for the chromatographic analysis: oven temperature of 60 °C; injection temperature of 250 °C; split injection mode; gas flow rate of 1.30 ml/min; carrier gas – nitrogen (N_2); heating ramp from 60 to 240 °C; heating increase rate of 3 °C/min; and the ionization source was electron impact (EI).

The Retention Indexes (RI) for the components of the volatile oils were calculated by the equation of Dool and Kratz (1963), using the retention times of the components of the oils and of the homologous series of alkanes (C_9–C_{25}), and the calculated indexes were then used to the identification of these components by comparing the obtained RI and mass spectra with those available in literature and in the following libraries: Flavours and Fragrances of Natural and Synthetic Compounds (FFNSC 1.3), Wiley® Mass Spectral Library, National Institute of Standards and Technology (NIST Webbook) and The Pherobase online.

Environmental data analysis

The environmental data used to carry out this study were divided into climate and soil data. The climate data consisted of rainfall, humidity, temperature and level of solar radiation, provided by the National Institute of Meteorology (Instituto Nacional de Meteorologia – Inmet). Regarding soil data, from December 2012, three collections were performed every six months, until December 2013, following the standard procedure for soil analysis recommended by the Agronomic Institute of Campinas (Instituto Agronômico de Campinas, IAC). Soil samples were placed in labelled plastic bags (four replicates for each geographical locations) and a 200 g aliquot was separated and sent for soil analysis of macro and micronutrients, including sulphur and aluminium composition (Silva, 2009).

Data processing and multivariate analysis

The data obtained after the analysis of the volatile oils by GC–MS (chemical data) and the climate and soil data (environmental data) were prior pre-treated in MS Excel© and used for further multivariate statistical analysis by the software CANOCO 4.5

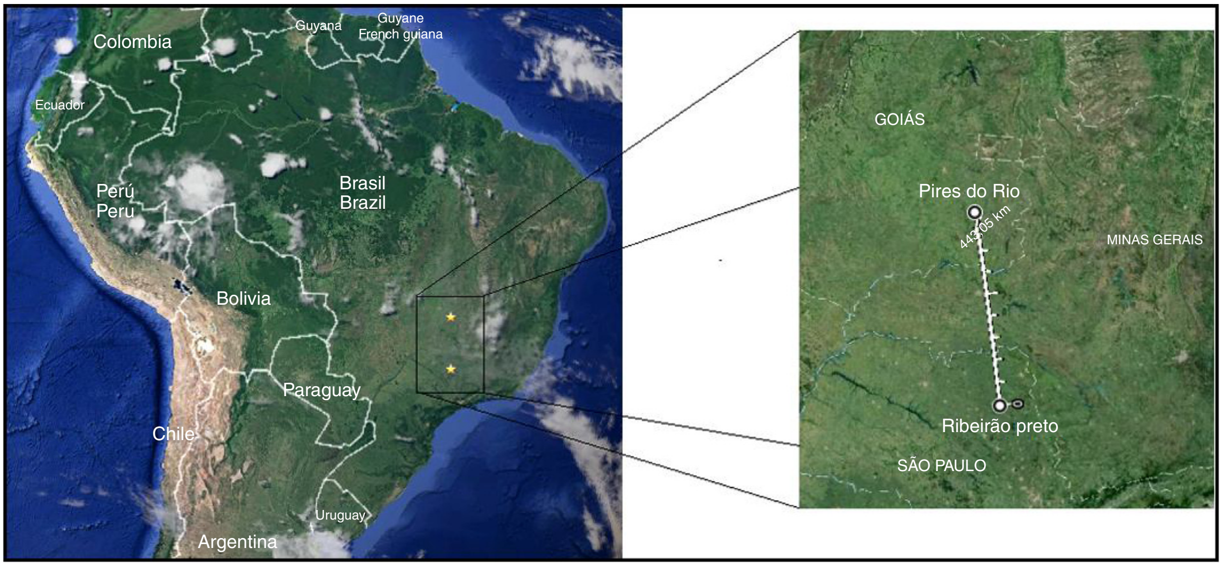

Fig. 1. Sampling locations of *Tithonia diversifolia* leaves and inflorescences from Brazil.

Source: Google Earth© (Google Inc.©).

(Biometris – Plant Research International©), OriginPro 9.6 (OriginLab Corporation©) and SIMCA-P (Umetrics©).

Prior to performing the multivariate analysis, the chemical data were transformed by the formula described in Eq. (1), so that x is the value of the peak area for each compound expressed as percentage. The environmental data were transformed according to Eq. (1), for the data expressed as percentage, such as BS (percent base saturation) and relative humidity, and according to Eq. (2) for the other environmental variables not expressed as percentage, so that x is the value of the individual observations for each variable. For the soil pH, unprocessed data were used. All the data from chemical and environmental variables were mean centred.

All the previous steps were performed in order to scale the data prior to the multivariate analysis.

$$\hat{x}_n = \arcsin\left(\sqrt{\frac{x_n}{100}}\right) \tag{1}$$

(1) Transformation of variables by the arcsin method.

$$\hat{x}_n = \log_{10}(x_n + 1) \tag{2}$$

(2) Logarithmic transformation for the variables.

The data for the study of variation in the composition of the volatile oils of *T. diversifolia* (chemical and environmental data), after appropriate treatment, were analyzed in the software CANOCO 4.5 (Biometris – Plant Research International©), OriginPro 9.6 (OriginLab Corporation©) and SIMCA-P (Umetrics©). The data were divided into two sets of variables: chemical (volatile oils) and environmental (climate and soil data) variables. These variables were used to perform multivariate analysis by unsupervised methods, such as Principal Component Analysis (PCA) and Hierarchical Cluster Analysis (HCA) by Ward's method (Ward, 1963), and by supervised methods as the Orthogonal Partial Least Squares-Discriminant Analysis (OPLS-DA).

Results and discussion

Environmental data

In Fig. 1 are shown the exact location and distance between both localities where the samplings of leaves and inflorescences of *T. diversifolia* were collected to obtain the volatile oil samples. The environmental data (climate and soil data) were obtained from both collection sites during the year of 2013. The climate data are shown in Fig. 2 and the soil in Tables 1 and 2.

The results of the soil analysis showed some differences among the two collection sites (located at two different Brazilian states), especially regarding to the levels of macronutrients P and Ca, so that the soil from the city of Pires do Rio, state of Goiás (GO), was richer in these minerals than the soil from the city of Ribeirão Preto, state of São Paulo (SP).

Climate data showed the differences between the local climates of both collection sites, with GO being warmer and drier than SP, showing higher levels of solar radiation, mainly during the dry season (white areas shown in Fig. 2). These differences between the local climates were expected considering that the city in GO is located in a lower latitude region than the city in SP (see Fig. 1).

Chemical variation of the volatile oils of leaves and inflorescences of Tithonia diversifolia

The chemical composition of the volatile oils of leaves and inflorescences of *T. diversifolia* obtained from samples collected at different seasons during the year of 2013 in GO and SP are shown in the Tables 3 and 4. The results are expressed as percentage of the constituents for each sample of volatile oil.

The dendrogram obtained by Hierarchical Cluster Analysis (HCA) from GC–MS data of the volatile oils from leaves and inflorescences of *T. diversifolia* is shown in Fig. 3. Based on the pattern of sample separation observed in the HCA dendrogram, two main clusters were proposed for the volatile oils of *T. diversifolia*, in accordance to the geographical origin of the samples.

The chemical (GC–MS) and environmental (climate and soil) data were used to carry out a Principal Component Analysis (PCA) with environmental variables. Fig. 4 shows the triplot graph obtained by the combined analysis of the chemical and environmental variables for the samples of volatile oils of *T. diversifolia*. It is possible to observe a good separation of the sample clusters, in the same way as it was observed in the dendrogram of HCA analysis (Fig. 3). In addition, the discriminant chemical constituents for the clusters (grey squares identified by the red numbers in Fig. 4) and the environmental factors (levels of

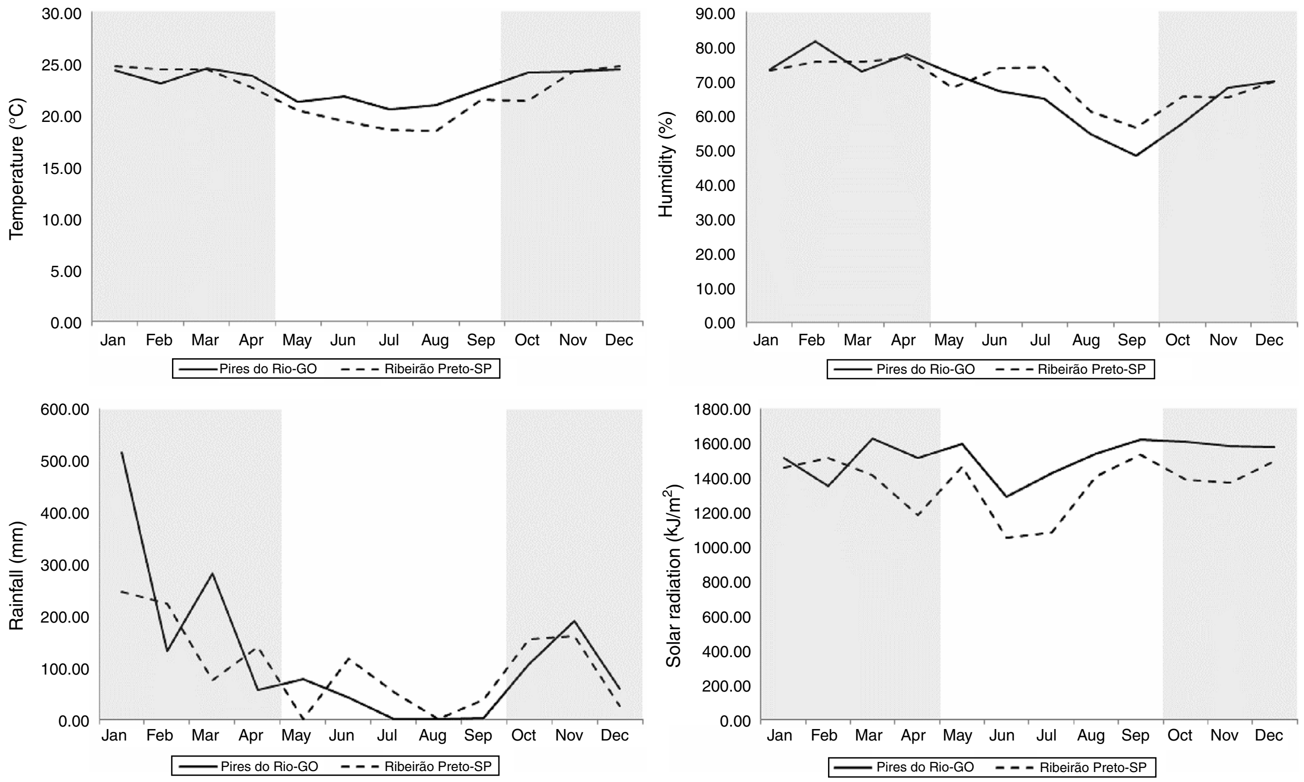

Fig. 2. Climate data obtained from both collection sites during the year of 2013. Temperature, humidity and solar radiation are expressed as monthly average values and rainfall is expressed as accumulated rain per month. Continuous line = Pires do Rio-GO; Dashed line = Ribeirão Preto-SP. Areas in grey indicate the dry season (typical flowering season) and areas in white indicate the rainy season (season typically without flowers).

Table 1

Levels of soil macro and micronutrients of the localities of Pires do Rio, state of Goiás (GO), and Ribeirão Preto, state of São Paulo (SP).

Sampling	P[a]	K[b]	Ca[b]	Mg[b]	S[a]	B[a]	Cu[a]	Fe[a]	Mn[a]	Zn[a]
GO-December/12	12.0	2.9	32.0	11.0	6.0	0.24	1.3	23.0	4.9	2.2
GO-June/13	13.0	1.8	34.0	7.0	5.0	0.20	1.2	22.0	1.9	0.6
GO-December/13	24.0	3.2	27.0	9.0	5.0	0.22	2.0	38.0	5.7	2.3
SP-December/12	12.0	2.8	27.0	9.0	7.0	0.24	4.1	10.0	10.7	0.4
SP-June/13	9.0	2.5	20.0	8.0	5.0	0.23	3.8	11.0	10.0	0.3
SP-December/13	7.0	2.6	22.0	7.0	6.0	0.20	5.2	14.0	13.6	0.6

[a] P, Fe, Mn, Cu, Zn, B and S expressed as mg/dm^3.

[b] K, Ca, Mg expressed as mmolc/dm^3.

Table 2

Other soil mineral composition indicators of the localities of Pires do Rio, state of Goiás (GO), and Ribeirão Preto, state of São Paulo (SP).

Sampling	pH ($CaCl_2$)	OM[a]	H + Al[b]	Al[b]	CEC[b]	TEB[b]	BS[c]
GO-December/12	5.6	23.0	22.0	0	67.0	45.0	67.9
GO-June/13	6.0	22.0	20.0	0	63.0	43.0	68.1
GO-December/13	5.5	25.0	21.0	0	60.0	39.0	64.9
SP-December/12	5.2	28.0	32.0	0	70.0	38.0	54.4
SP-June/13	5.1	27.0	34.0	0	65.0	31.0	47.5
SP-December/13	5.3	25.0	29.0	0	60.0	32.0	52.6

[a] OM expressed as g/dm^3.

[b] CEC, H + Al, Al and TEB expressed as mmolc/dm^3.

[c] BS expressed as percentage. Legend: OM, organic matter; H + Al, value of potential acidity of soil; CEC, cation exchange capacity; TEB, total exchangeable bases; BS, base saturation of soil.

soil Ca and P; temperature and solar radiation) that exert influence on the production of volatile oils in *T. diversifolia* could also be observed. The constituents of the volatile oils identified as discriminants (identified with numbers in Fig. 4) are the following monoterpenes 1 (α-pinene), 2 (β-pinene), and 3 (*cis*-chrysanthenol); and the sesquiterpenes 4 (β-caryophyllene), 5 (bicyclogermacrene), 6 (kessane), 7 (caryophyllene oxide), and 8 (ledene oxide).

The chemical data of the volatile oils were submitted to analysis by Orthogonal Partial Least Square-Discriminant Analysis (OPLS-DA) in order to evaluate the separation of the samples according to the clusters already proposed by the HCA analysis. The score scatter plot of the OPLS-DA analysis is shown in Fig. 5, demonstrating the proposed clusters well separated among each other, therefore in accordance to the results obtained by unsupervised methods – HCA and PCA, which confirm the reliability of these results. There is only an outlier sample (not grouped into the cluster of its collection site), the volatile oil obtained from samples of leaves collected on January 2013 in the city of Ribeirão Preto-SP.

Table 5 shows the coefficient values to each constituent of the volatile oils of *T. diversifolia* for the first two axes of PCA loading scatter plot (Axis 1 and Axis 2). Table 5 shows the main discriminant constituents related to each cluster of volatile oil samples, based on the results of PCA and OPLS-DA analyses. The coefficient values for the first axis of α- and β-pinene (0.5057 and 0.8172, respectively) show that both monoterpenes are the most important discriminant constituents for the volatile oil samples from Goiás and São Paulo. The samples can be clearly separated in two large and distinct geographical clusters (see Fig. 4), according to their levels of α-pinene (Goiás) and β-pinene (São Paulo). The coefficient values for the second axis of other constituents highlighted in Table 5 and graphical results showed in Fig. 4 demonstrate that *cis*-chrysanthenol is the main discriminant constituent of the inflorescence volatile oils, distinguishing them from the leaf volatile oils. In the same way, the sesquiterpenes bicyclogermacrene, β-caryophellene, caryophellene oxide, kessane and ledene oxide are the main discriminant constituents of the leaf volatile oils from Goiás.

Table 3
Chemical constituents (expressed as percentage) of the volatile oils obtained from samples of leaves of *Tithonia diversifolia* collected at different seasons of the year of 2013 in the regions of Pires do Rio-GO and Ribeirão Preto-SP.

Compounds	RI	Pires do Rio-GO						Ribeirão Preto-SP					
		January	May	June	July	August	December	January	May	June	July	August	December
Tricyclene	922	–	0.51	0.43	–	0.33	0.19	–	0.18	0.34	0.16	0.29	0.29
α-Thujene	924	–	0.42	0.31	–	0.16	0.15	–	0.16	0.13	0.13	–	0.24
α-Pinene	932	17.82	41.92	32.58	35.35	53.65	55.55	75.53	26.20	27.31	31.21	36.98	39.52
Camphene	948	4.25	7.55	6.34	2.64	5.71	2.18	2.92	2.87	5.84	2.58	5.51	2.49
Sabinene	971	4.08	4.51	3.46	1.49	2.16	2.01	2.41	3.36	2.61	2.33	2.44	2.60
β-Pinene	977	2.75	2.93	2.29	1.10	2.34	1.25	1.57	49.44	39.20	32.18	39.05	41.64
5-Hexenyl-oxirane	996	–	–	–	–	–	–	–	–	–	–	–	0.05
α-Terpinene	1015	–	–	–	–	–	0.04	–	–	–	–	–	–
p-Cymene	1022	–	0.31	–	–	–	0.06	–	–	–	–	–	0.03
Limonene	1027	7.78	8.63	3.27	1.74	2.09	3.41	3.83	3.80	2.97	2.82	2.72	3.21
β-Phellandrene	1029	–	0.56	0.14	–	0.18	0.22	–	1.13	0.83	1.01	1.05	0.98
cis-β-Ocimene	1033	4.09	3.27	4.27	2.71	2.38	2.44	2.17	1.19	1.06	1.58	0.66	0.79
γ-Terpinene	1056	–	0.47	–	–	–	0.12	–	–	–	0.15	–	–
trans-Sabinene hydrate	1069	0.83	0.39	–	–	0.29	0.22	–	0.32	0.32	0.21	–	0.04
Linalool	1098	–	–	–	–	–	–	–	–	0.20	–	–	0.18
RI 1099	1099	–	–	–	–	–	–	–	–	–	–	–	0.03
cis-Sabinene hydrate	1101	0.66	0.37	–	–	0.31	0.17	–	0.42	0.38	0.40	–	–
trans-Verbenol	1143	0.64	–	–	–	–	–	–	–	–	–	–	–
cis-Verbenol	1144	–	–	–	–	–	0.13	–	–	0.35	–	–	–
cis-Chrysanthenol	1161	–	–	–	–	–	–	–	0.87	0.45	0.76	0.44	0.11
Borneol	1170	6.54	4.59	0.48	2.37	4.05	2.24	2.04	2.69	6.37	2.92	4.26	1.49
Terpinen-4-ol	1179	1.12	1.11	–	–	–	0.37	–	0.61	0.62	0.40	–	0.12
trans-3(10)-Caren-2-ol	1211	–	–	–	–	–	–	–	0.25	–	0.33	–	–
2-Methyl-2-nonen-4-one	1211	–	–	–	–	–	0.13	–	–	–	–	–	–
Bicycloelemene	1329	0.64	–	–	–	–	–	–	–	–	–	–	–
α-Copaene	1372	3.55	2.14	3.18	2.57	3.56	2.04	0.93	0.64	0.63	1.05	0.34	0.74
Modhephene	1377	–	–	0.74	0.61	0.53	0.15	–	–	–	–	–	–
RI 1379	1379	–	–	–	–	–	–	–	–	–	–	–	0.08
RI 1381	1381	–	–	–	–	–	–	–	–	0.60	–	–	–
RI 1384	1384	–	–	–	–	–	0.11	–	–	–	–	–	–
RI 1391	1391	–	–	–	–	–	–	–	–	–	–	–	0.11
RI 1393	1393	–	–	–	–	–	–	–	–	0.37	–	–	–
β-Caryophyllene	1414	8.16	4.71	16.08	8.15	5.21	6.87	2.28	1.18	3.09	5.15	1.39	1.31
α-Humulene	1450	–	–	0.81	0.43	–	0.29	–	–	–	0.27	–	–
Germacrene D	1476	–	–	0.44	–	–	0.29	–	–	–	–	–	–
Bicyclogermacrene	1490	8.72	3.29	10.57	6.51	5.00	9.35	1.97	0.76	0.88	4.95	0.45	1.07
1-Pentadecene	1492	–	–	1.82	1.94	1.45	–	–	–	0.31	0.25	–	–
RI 1498	1498	–	–	–	–	–	0.20	–	–	–	–	–	–
δ-Cadinene	1514	–	–	0.65	0.51	0.32	0.42	–	–	–	–	–	–
Kessane	1523	5.05	2.91	3.48	5.41	3.15	1.92	1.13	–	0.25	–	–	–
RI 1529	1529	–	–	–	0.79	–	–	–	–	–	–	–	–
RI 1530	1530	–	–	0.42	–	–	0.23	–	–	–	–	–	–
trans-Nerolidol	1559	2.32	0.93	0.62	1.23	0.48	0.70	–	–	0.58	0.73	–	0.33
Spathulenol	1572	7.76	2.23	1.72	5.86	1.05	2.08	1.40	1.14	0.95	2.01	1.02	0.87
Caryophyllene Oxide	1575	5.88	3.27	3.01	7.55	1.87	1.54	0.91	1.09	1.31	2.16	1.21	0.56
Ledene Oxide	1578	5.34	2.18	1.47	4.35	1.56	1.96	0.91	1.10	0.91	2.13	1.08	0.78
RI 1592	1592	–	–	–	–	–	0.12	–	–	–	–	–	–
RI 1598	1598	0.86	–	–	0.90	–	0.28	–	–	–	0.38	–	0.09
RI 1601	1602	–	–	–	0.53	–	–	–	–	–	–	–	–
RI 1608	1608	–	–	–	0.39	–	0.14	–	–	–	–	–	–
RI 1627	1627	–	–	–	0.47	–	–	–	–	–	–	–	–
11,11-Dimethyl-4,8-bis(methylene)-bicyclo[7.2.0]undecan-3-ol	1631	–	–	–	0.71	–	–	–	–	–	–	–	–
Intermedeol	1652	1.17	0.78	1.41	3.70	2.19	0.45	–	0.60	1.14	1.77	1.10	0.26
Total (%)		100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

The results of the analysis of the volatile oils of *T. diversifolia* obtained in different seasons by GC–MS provided an overview of the main volatile components present in the leaves and inflorescences of this species. It was observed the predominance of monoterpenes as major components in all the analyzed samples (mainly hydrocarbon monoterpenes). It is also worth mentioning the presence in some volatile oil samples (even in small proportion) of some metabolites that do not belong to the group of terpenes, such as fatty acids, aldehydes, cyclic ethers and hydrocarbons, which, in their vast majority, were found in samples of volatile oils of the inflorescences.

In general, the observed difference in the composition between the volatile oils of leaves and inflorescences is not reflected in a difference with respect to their main constituents, which are the monoterpenes α- and β-pinene. The only exception with respect to β-pinene as a major component in the volatile oil are the samples obtained from leaves collected in GO, in which only the α-pinene is presented as one of the major components, along with some sesquiterpenes (hydrocarbon and oxygenated) in a higher proportion in the oil, in particular β-caryophyllene, caryophyllene oxide, bicyclogermacrene, kessane and ledene oxide.

Table 4
Chemical constituents (expressed as percentage) of the volatile oils obtained from samples of inflorescences of *Tithonia diversifolia* collected at different seasons of the year of 2013 in the regions of Pires do Rio-GO and Ribeirão Preto-SP.

Compounds	RI	Pires do Rio-GO				Ribeirão Preto-SP		
		May	June	July	December	May	June	July
Tricyclene	922	–	0.50	–	0.12	–	–	–
α-Thujene	924	0.34	0.46	–	0.16	0.32	0.31	–
α-Pinene	932	44.26	57.18	88.20	85.67	33.80	36.30	33.83
Camphene	948	0.91	1.77	–	0.59	0.86	1.37	0.64
2,4-Thujadiene	951	0.31	–	–	–	–	–	–
Sabinene	971	–	1.88	–	0.95	0.68	1.27	0.92
β-Pinene	976	0.84	1.39	–	0.56	37.37	38.26	31.27
2-Pentylfuran	989	0.98	–	–	–	0.22	0.48	0.09
5-Hexenyl-oxirane	996	1.15	0.60	–	0.27	1.99	0.50	1.07
α-Terpinene	1016	–	–	–	–	0.20	–	–
m-Cymene	1024	0.20	–	–	–	–	–	–
Limonene	1027	1.88	3.25	1.86	2.22	3.24	2.91	2.86
RI 1029	1029	–	–	–	0.01	–	–	–
β-Phellandrene	1029	–	–	–	–	0.22	0.27	0.34
cis-β-Ocimene	1033	1.49	4.12	–	0.49	0.86	0.83	0.73
γ-Terpinene	1056	0.40	–	–	0.11	0.39	0.20	–
α-Terpinolene	1084	0.41	–	–	–	0.39	–	–
cis-6-Nonenal	1097	–	–	–	–	–	–	0.26
Linalool	1100	5.19	1.84	–	0.24	1.43	0.31	1.09
trans-4,8-Dimethyl-1,3,7-nonatriene	1111	1.13	1.56	–	0.51	–	–	0.40
cis-4,8-Dimethyl-1,3,7-nonatriene	1113	–	–	–	–	0.48	–	–
2-Methyl-6-methylene-1,7-octadien-3-one	1113	–	–	–	–	–	0.46	–
RI 1149	1149	0.57	–	–	–	–	–	–
cis-Chrysanthenol	1161	10.69	4.10	4.14	0.75	4.43	1.43	4.16
Borneol	1170	3.10	0.73	–	–	1.03	–	0.93
Terpinen-4-ol	1179	1.45	–	–	0.09	0.86	–	0.42
α-Terpineol	1194	2.90	–	–	–	1.71	–	0.96
Isoborneol	1202	0.76	–	–	–	–	–	–
RI 1245	1245	0.39	–	–	–	–	–	–
α-Copaene	1372	8.24	8.33	5.79	3.38	5.97	4.80	7.24
Modhephene	1377	0.77	0.78	–	0.20	–	–	0.58
β-Caryophyllene	1414	–	–	–	–	0.39	0.65	0.36
Germacrene D	1476	–	–	–	0.05	–	–	–
trans-α-Bergamotene	1489	0.99	–	–	0.15	–	–	–
trans-β-Bergamotene	1490	–	0.92	–	–	–	–	0.30
1-Pentadecene	1492	1.31	2.14	–	0.75	0.92	0.88	1.49
δ-Cadinene	1514	1.00	0.73	–	0.37	0.39	–	0.39
Kessane	1523	1.98	1.47	–	0.38	–	–	–
trans-Nerolidol	1559	0.57	–	–	–	–	–	–
Spathulenol	1571	1.54	1.39	–	0.53	1.34	1.12	1.28
β-Copaen-4-α-ol	1582	–	–	–	–	–	–	0.30
Intermedeol	1652	0.74	–	–	0.11	0.52	1.24	0.95
Pentadecanal	1714	0.67	–	–	–	–	–	–
Palmitic acid	1961	–	–	–	0.17	–	0.82	0.67
RI 2029	2029	–	–	–	0.38	–	–	–
Falcarinol	2029	–	1.43	–	–	–	2.11	3.15
Tricosane	2300	–	1.28	–	–	–	1.24	1.41
RI 2300	2300	–	–	–	0.37	–	–	–
Eicosenoic acid	2300	1.08	–	–	–	–	–	–
RI 2370	2370	1.36	1.45	–	0.37	–	2.26	0.84
Pentacosane	2500	0.42	0.69	–	–	–	–	1.06
Total %		100.0	100.0	100.0	100.0	100.0	100.0	100.0

The chemical composition of the volatile oils of leaves and inflorescences of *T. diversifolia* is well described in literature with results that demonstrate differences in the proportion of the major constituents among samples from different geographical regions, especially the α-pinene, which proportion ranges from 25.0 to 60.9% and from 6.8 to 72.8%, in leaves and inflorescences, respectively (Lamaty et al., 1991; Menut et al., 1992; Moronkola et al., 2007; Lawal et al., 2012; Dai et al., 2015; Orsomando et al., 2016). These data from literature combined with the results obtained herein by HCA and PCA analyses (Figs. 3 and 4, respectively) indicate that the surrounding environment seems to significantly influence the production and accumulation of volatile constituents in aerial parts of *T. diversifolia*.

The existence of chemotypes for plants producing volatile oils is common and well reported in the literature for different species, especially for those used for food, medicinal purposes or in perfumery (Vokou et al., 1993; Boira and Blanquer, 1998; Curado et al., 2006; Telci et al., 2006; Costa et al., 2009; Satyal et al., 2016). An example is the study performed with samples from different populations of *Lippia origanoides* Kunth, Verbenaceae, collected in Colombia, which led to the characterization of three different chemotypes for the species according to the composition of its volatile oil (Stashenko et al., 2010). Another example of aromatic plants that show different chemotypes is the species *Origanum syriacum* L., Lamiaceae, which, after study performed with samples from eleven populations in Syria, demonstrated the existence of three different chemotypes based on the composition of the volatile oils (Lukas et al., 2009).

For the specimens of *T. diversifolia* evaluated in this work, we propose the existence of two chemotypes according to the

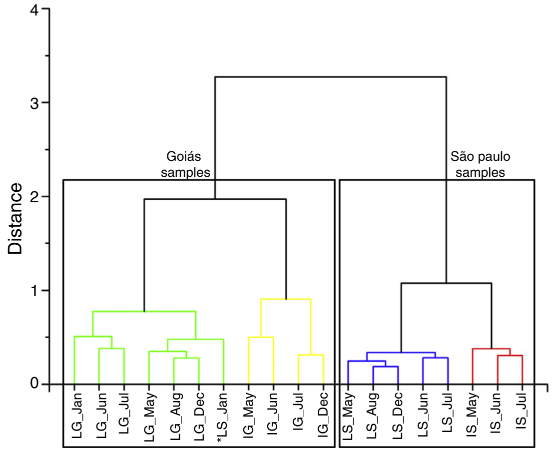

Fig. 3. HCA dendrogram for GC–MS data obtained from volatile oils of leaves and inflorescences of *T. diversifolia* with the clustering proposal. L, Leaves; I, Inflorescences; G, State of Goiás; S, State of São Paulo. *Outlier sample (not grouped with the others from the same collection site).

geographical origin in Brazil: a chemotype for the samples from the locality of Pires do Rio, state of Goiás (chemotype PR-GO), and another for the locality of Ribeirão Preto, state of São Paulo (chemotype RP-SP). The chemotype PR-GO, based on results of PCA analysis, can be characterized by the higher proportion of the constituents bicyclogermacrene, β-caryophellene, caryophyllene oxide, kessane and ledene oxide in leaf volatile oil, and α-pinene and *cis*-chrysanthenol in inflorescence volatile oil. The chemotype RP-SP is characterized by the higher proportion of the constituent β-pinene in both leaf and inflorescence volatile oils, plus *cis*-chrysanthenol only in inflorescence volatile oil.

It is noteworthy that the monoterpene β-pinene is described for the first time as the most abundant constituent in samples of volatile oil of leaves and inflorescences of *T. diversifolia*, once in all previous studies carried out with the volatile oils from this species, the content of β-pinene in the oil samples was much lower than that of α-pinene. This result provides preliminary evidence of the existence of a chemotype which allows to characterize differently these samples of volatile oils obtained from leaves and inflorescences collected in SP, based on the β-pinene proportion.

The result of the PCA analysis with environmental data (Fig. 4) indicates a clear relationship between the two chemotypes observed for the volatile oils of *T. diversifolia* and their geographical origin, so that in our study, the main environmental factors related to the variation of the volatile oils of *T. diversifolia* are the soil macronutrients Ca and P, the temperature and solar radiation level.

The levels of the macronutrients Ca and P in the soil appear to exert some influence on the composition of the volatile oils of leaves and inflorescences of *T. diversifolia*. Thus, the oil samples obtained by specimens located at the soil richer in these nutrients (GO) showed α-pinene as the main constituent of the volatile oils, with low levels of β-pinene. On the other hand, the oil samples obtained from leaves and inflorescences collected from specimens located in the soil with lower levels of Ca and P (SP) showed both α-pinene and β-pinene as the main constituents, with similar content for the both monoterpenes in the volatile oils throughout the year, except for the leaf oil sample obtained in January 2013.

The presence of α- and β-pinene is very common in volatile oils of plants, occurring in a wide range of tropical, Mediterranean and coniferous species (Areco et al., 2014). In general, these

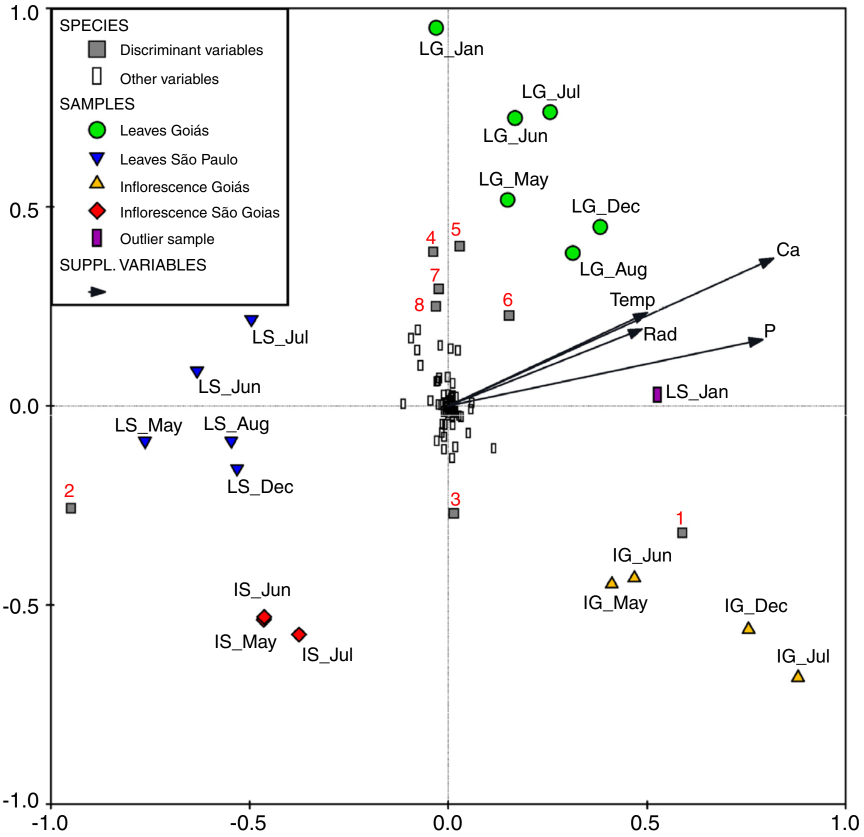

Fig. 4. PCA triplot for the GC–MS data obtained from the volatile oils of leaves and inflorescences of *T. diversifolia* collected during the year of 2013 and the relationship with the environmental factors. L, leaves; I, inflorescences; G, State of Goiás; S, State of São Paulo; Ca, Soil calcium; P, Soil phosphorus.

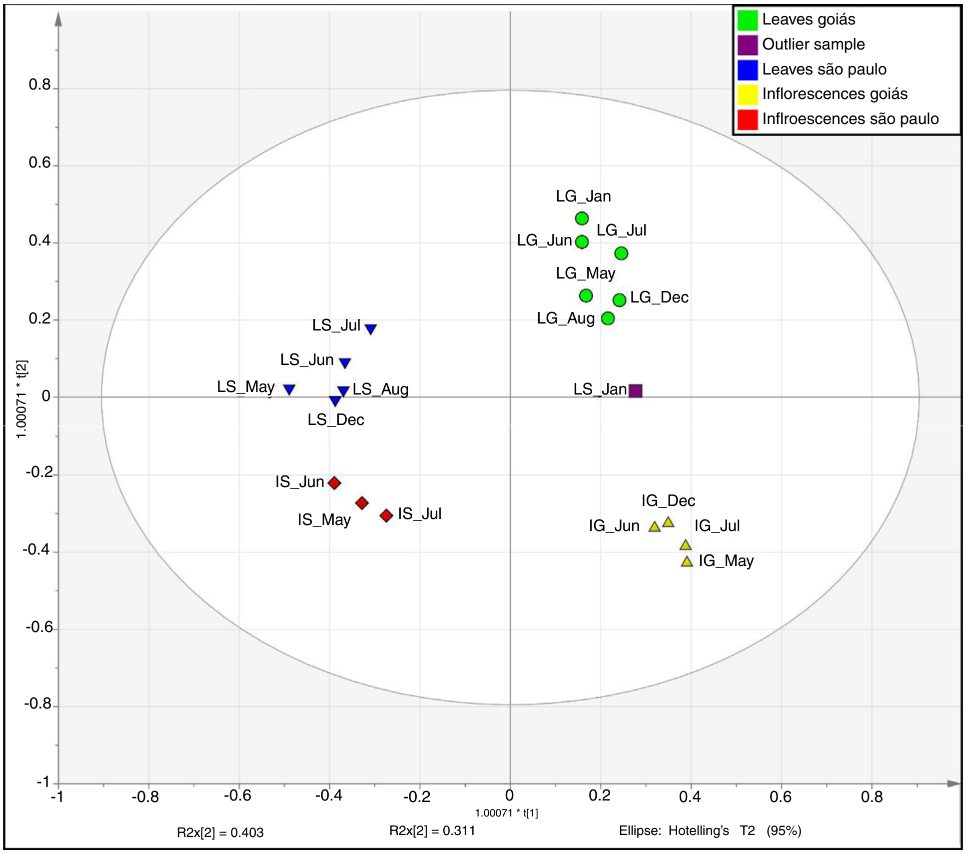

Fig. 5. OPLS-DA score scatter plots for the GC–MS data obtained from volatile oils of leaves and inflorescences of *T. diversifolia* collected during the year of 2013. L, Leaves; I, Inflorescences; G, State of Goiás; S, State of São Paulo.

monoterpenes are involved in allelopathic interactions among plant species, once they show phytotoxic effects as the inhibition of the germination and growth of other plants (Abrahim et al., 2003; Scrivanti et al., 2003; Nishida et al., 2005; Singh et al., 2006, Kegge and Pierik, 2010; Chowhan et al., 2011; Amri et al., 2012).

The Mexican sunflower shows allelopathic potential over other species related to the production of different secondary metabolites, including phenolics and terpenes (Baruah et al., 1994; Tongma et al., 1998, 2001; Ademiluyi, 2012, 2013; Adesina, 2013; Miranda et al., 2015), which represents a great adaptive advantage for an invasive weed against competitors under different environmental conditions by the decreasing of the competition for some volatile resources as soil nutrients, water and light (Miranda et al., 2015).

The higher content of β-pinene in the leaf and inflorescence volatile oils of the samples from SP in comparison with the samples from the region of GO can be related to the allelopathic effect of this monoterpene and the availability of Ca and P in the soils of both regions. Some studies indicate that β-pinene is more phytotoxic than α-pinene (Nishida et al., 2005; Chowhan et al., 2011; Areco et al., 2014) and that the production and emission of biogenic volatile organic compounds (BVOC) of plants can be affected by competition for limited resources as soil nutrients and light (Kegge and Pierik, 2010). Therefore, the high content of β-pinene in the samples of SP might be a responsive mechanism to the low levels of soil Ca and P, through the increasing of a more potent phytotoxic compound (β-pinene) aiming to decrease the competition by other species for soil nutrients.

The higher content of sesquiterpenes, mainly bicyclogermacrene, β-caryophellene, caryophyllene oxide and kessane, in the leaf volatile oil samples from GO can be possibly linked to genetic factors of the population in addition to the combination of the environmental factors of the region during the period of study (high Ca and P levels in soil combined with higher average temperature and solar radiation levels).

The high average temperature and high levels of solar radiation in GO seem to be related to the increased production of sesquiterpenes in leaf volatile oil samples from this location, since the effects of these factors in the composition of plant volatile oils is well described. According to Rosenfeld et al. (2002), the production and emission of sesquiterpenes, especially β-caryophellene, was increased the in volatile fraction obtained from roots of *Daucus carota* L., Apiaceae, under high temperature conditions, and a similar result is described by Vallat et al. (2005) for the volatile fraction of apple trees. Solar radiation also can be related to an increased accumulation of sesquiterpenes in plant volatile oil, as described in a study carried out using leaves of *Pothomorphe umbellatum* L., Piperaceae, which shows that the proportion of sesquiterpenes, especially the major component of the volatile oil, *trans*-nerolidol, was higher in plants cultivated under full sun compared to plants cultivated under different shade conditions (Mattana et al., 2010).

The reliable results reported herein concerning the chemical composition of volatile oils from leaves and inflorescences, along with the data described elsewhere, provide new evidences of the existence of different chemotypes for the species *T. diversifolia* according to the geographical origin of the samples. The differences observed in the production and accumulation of some constituents of the volatile oils, especially the major constituents, seem to be related, but not exclusively, to changes in abiotic environmental factors, as soil nutrients and climate, although the influence of genetic and biotic environmental factors on the composition of volatile oils should also be considered. These results, especially regarding the difference in accumulation of β-pinene in the leaf

Table 5
Coefficient values of the chemical constituents from the volatile oils of *T. diversifolia* for the first two axes of the PCA loading scatter plot (Axis 1 and Axis 2).

Compounds[a]	Axis 1	Axis 2
Tricyclene	0.01027	0.02721
α-Thujene	0.005567	0.01166
α-Pinene	**0.5057**	**0.3156**
Camphene	0.06617	0.1879
2,4-Thujadiene	0.007417	0.00928
Sabinene	0.06783	0.139
β-Pinene	**0.8172**	**0.2534**
2-Pentylfuran	0.007924	0.04599
5-Hexenyl-oxirane	0.009651	0.107
α-Terpinene	0.004265	0.005593
p-Cymene	0.002728	0.01383
m-Cymene	0.005956	0.007453
Limonene	0.02027	0.06926
RI 1029	0.002445	0.00209
β-Phellandrene	0.1027	0.00487
cis-β-Ocimene	0.02015	0.1379
γ-Terpinene	0.001782	0.01662
trans-Sabinene hydrate	0.02304	0.06243
α-Terpinolene	0.000883	0.02317
cis-6-Nonenal	0.006214	0.01091
Linalool	0.007565	0.1288
RI 1099	0.002984	0.001012
cis-Sabinene hydrate	0.02597	0.0604
trans-4,8-Dimethyl-1,3,7-nonatriene	0.04292	0.06639
cis-4,8-Dimethyl-1,3,7-nonatriene	0.01044	0.01386
2-Methyl-6-methylene-1,7-octadien-3-one	0.01018	0.01339
trans-Verbenol	0.000805	0.02833
cis-Verbenol	0.007667	0.007974
RI 1149	0.01006	0.01259
***cis*-Chrysanthenol**	**0.01151**	**0.2663**
Borneol	0.08113	0.1686
Terpinen-4-ol	0.03855	0.01236
α-Terpineol	0.008927	0.07571
Isoborneol	0.01162	0.01454
trans-3(10)-Caren-2-ol	0.0216	0.003019
2-Methyl-2-nonen-4-one	0.004461	0.006039
RI 1245	0.00832	0.01041
Bicycloelemene	0.000805	0.02833
α-Copaene	0.09795	0.1058
Modhephene	0.05008	0.007083
RI 1379	0.004873	0.001652
RI 1381	0.01589	0.002533
RI 1384	0.004103	0.005555
RI 1391	0.005714	0.001937
RI 1393	0.01247	0.001989
β-Caryophyllene	**0.03342**	**0.3833**
α-Humulene	0.008648	0.05557
Germacrene D	0.01574	0.02224
trans-α-Bergamotene	0.02274	0.0247
trans-β-Bergamotene	0.007878	0.0272
Bicyclogermacrene	**0.02381**	**0.3973**
1-Pentadecene	0.02697	0.02675
RI 1498	0.005533	0.007492
δ-Cadinene	0.04822	0.008682
Kessane	**0.1311**	**0.2239**
RI 1529	0.00737	0.02447
RI 1530	0.009456	0.02552
trans-Nerolidol	0.004802	0.1419
Spathulenol	0.01793	0.1507
Caryophyllene oxide	**0.02108**	**0.291**
Ledene oxide	**0.02761**	**0.247**
β-Copaen-4-α-ol	0.006676	0.01172
RI 1592	0.004286	0.005802
RI 1598	0.001586	0.07109
RI 1601	0.006034	0.02003
RI 1608	0.009804	0.02345
RI 1627	0.005682	0.01886
11,11-Dimethyl-4,8-bis(methylene)-bicyclo[7.2.0]undecan-3-ol	0.006986	0.0232
Intermedeol	0.06079	0.09996
Pentadecanal	0.01091	0.01365
Palmitic acid	0.0135	0.04403
RI 2029	0.01508	0.01289
Falcarinol	0.02545	0.08623
Tricosane	0.01406	0.06573
RI 2300	0.01488	0.01272
Eicosenoic acid	0.01386	0.01734
RI 2370	0.01491	0.101
Pentacosane	0.008669	0.04626

[a] The chemical constituents with coefficient values higher than 0.2 are highlighted in bold and underlined.

volatile oil according to the environmental conditions, are very interesting from the ecological point of view and suggest that *T. diversifolia* may use volatile secondary metabolites as allelochemicals.

The results presented herein for the volatile constituents of the leaves and inflorescences of *T. diversifolia* demonstrate that the variation in production of certain classes of secondary metabolites, such as the terpenes, seems to be a direct response to different abiotic environmental conditions and combined to the results from a study about the influence of the environment on the metabolic profile of different tissues of *T. diversifolia* (Sampaio et al., 2016), indicate that the tissue-specific alterations in its secondary metabolism might be a type of adaptive strategy to different environments.

Our study can pave the way for a further and more detailed analysis of *T. diversifolia* for a better understanding of the ecological characteristics of this species and, to the best of our knowledge, we describe for the first time a direct relationship between environment and production of the volatile oils from leaves and inflorescences of the species.

Authors contributions

BLS (PhD student) contributed in collecting plant samples and taxonomic identification, confection of herbarium samples, running the laboratory work, analysis of the data and drafted the paper. FBC designed the study, supervised the laboratory work and contributed to critical reading of the manuscript. All the authors have read the final manuscript and approved the submission.

Conflicts of interest

The authors declare that they do not have any conflict of interest.

Acknowledgments

The authors thank F.A. dos Santos and G.F. Padilla-Gonzállez (AsterBioChem, FCFRP-USP) for the valuable support with the collection and obtainment of the volatile oil samples of the individuals from SP, the laboratory technician Mrs. I.C.C. Turatti (Center for Mass Spectrometry of Organic Micromolecules, FCFRP-USP) to perform GC–MS analysis of the volatile oil samples, and W.R. de Souza for the assistance with the English revision of the text. Authors also thank the São Paulo Research Foundation – FAPESP (grant #2011/13361-6), CAPES and CNPq for the financial support.

References

Abrahim, D., Francischini, A.C., Pergo, E.M., Kelmer-Bracht, A.M., Ishii-Iwamoto, E.L., 2003. Effects of α-pinene on the mitochondrial respiration of maize seedlings. Plant Physiol. Biochem. 41, 985–991.

Ademiluyi, B.O., 2013. Investigation on the allelopathic effect of *Tithonia diversifolia* (Hemsl) (Mexican Sunflower) on *Tridax procumbens* (L). Caribb. J. Sci. Technol. 1, 224–227.

Ademiluyi, B.O., 2012. Effect of *Tithonia diversifolia* (Hemsl) A. Gray on the growth and yield of okra (*Abelmuscus esculentus*). J. Agric. Sci. Technol. B 2, 219–222.

Adesina, G.O., 2013. Does soil under natural *Tithonia diversifolia* vegetation inhibit seed germination of weed species? Am. J. Plant Sci. 4, 2165–2173.

Ajao, A.A., Moteetee, A.N., 2017. *Tithonia diversifolia* (Hemsl) A. Gray (Asteraceae: Heliantheae), an invasive plant of significant ethnopharmacological importance: a review. S. Afr. J. Bot. 113, 396–403.

Amri, I., Gargouri, S., Hamrouni, L., Hanana, M., Fezzani, T., Jamoussi, B., 2012. Chemical composition, phytotoxic and antifungal activities of *Pinus pinea* essential oil. J. Pest Sci. 85, 199–207.

Anvisa, 2010. Determinação de óleos voláteis em drogas vegetais. In: Farmacopeia Brasileira. Agência Nacional de Vigilência Sanitária, Brasília, pp. 195–196.

Areco, V.A., Figueroa, S., Cosa, M.T., Dambolena, J.S., Zygadlo, J.A., Zunino, M.P., 2014. Effect of pinene isomers on germination and growth of maize. Biochem. Syst. Ecol. 55, 27–33.

Ayeni, A.O., Lordbanjou, D.T., Majek, B.A., 1997. *Tithonia diversifolia* (Mexican sunflower) in south-western Nigeria: occurrence and growth habit. Weed Res. 37, 443–449.

Baruah, N.C., Sarma, J.C., Barua, N.C., Sarma, S., Sharma, R.P., 1994. Germination and growth inhibitory sesquiterpene lactones and a flavone from *Tithonia diversifolia*. Phytochemistry 36, 29–36.

Boira, H., Blanquer, A., 1998. Environmental factors affecting chemical variability of essential oils in *Thymus piperella* L. Biochem. Syst. Ecol. 26, 811–822.

Chagas-Paula, D.A., Oliveira, R.B., Rocha, B.A., Da Costa, F.B., 2012. Ethnobotany, chemistry, and biological activities of the genus *Tithonia* (Asteraceae). Chem. Biodivers. 9, 210–235.

Chowhan, N., Singh, H.P., Batish, D.R., Kohli, R.K., 2011. Phytotoxic effects of β-pinene on early growth and associated biochemical changes in rice. Acta Physiol. Plant 33, 2369–2376.

Costa, D.P., Santos, S.C., Seraphin, J.C., Ferri, P.H., 2009. Seasonal variability of essential oils of *Eugenia uniflora* leaves. J. Braz. Chem. Soc. 20, 1287–1293.

Curado, M.A., Oliveira, C.B.A., Jesus, J.G., Santos, S.C., Seraphin, J.C., Ferri, P.H., 2006. Environmental factors influence on chemical polymorphism of the essential oils of *Lychnophora ericoides*. Phytochemistry 67, 2363–2369.

Dai, D.N., Thang, T.D., Ogunmoye, A., Eresanya, O.I., Ogunwande, I.A., 2015. Chemical constituents of essential oils from the leaves of *Tithonia diversifolia, Houttuynia cordata* and *Asarum glabrum* grown in Vietnam. Am. J. Essent. Oils Nat. Prod. 2, 17–21.

Dool, H.V.d., Kratz, P.D., 1963. A generalization of the retention index system including linear temperature programmed gas–liquid partition chromatography. J. Chromatogr. A 2, 463–471.

Figueiredo, A.C., Barroso, J.G., Pedro, L.G., Scheffer, J.J.C., 2008. Factors affecting secondary metabolite production in plants: volatile components and essential oils. Flavour Fragr. J. 23, 213–226.

Gutbrodt, B., Dorn, S., Unsicker, S.B., Mody, K., 2012. Species-specific responses of herbivores to within-plant and environmentally mediated between-plant variability in plant chemistry. Chemoecology 22, 101–111.

Jones, O.A.H., Maguire, M.L., Griffin, J.L., Dias, D.a., Spurgeon, D.J., Svendsen, C., 2013. Metabolomics and its use in ecology. Austral Ecol. 38, 713–720.

Kegge, W., Pierik, R., 2010. Biogenic volatile organic compounds and plant competition. Trends Plant Sci. 15, 126–132.

Lamaty, G., Menut, C., Zollo, P.-H.A., Kuiate, J.R., Bessière, J.-M., Koudou, J., 1991. Aromatic plants of tropical Central Africa. III. Constituents of the essential oil of the leaves of *Tithonia diversifolia* (Hemsl.) A. Gray from Cameroon. J. Essent. Oil Res. 3, 399–402.

Lawal, O.A., Kasali, A.A., Opoku, A.R., Oyedeji, A.O., 2012. Volatile constituents of the flowers, leaves, stems and roots of *Tithonia diversifolia* (Hemsely) A. Gray. J. Essent. Oil Bear Plants 15, 816–821.

Lukas, B., Schmiderer, C., Franz, C., Novak, J., 2009. Composition of essential oil compounds from different Syrian populations of *Origanum syriacum* L. (Lamiaceae). J. Agric. Food Chem. 57, 1362–1365.

Mattana, R.S., Vieira, M.A.R., Marchese, J.A., Ming, L.C., Marques, M.O.M., 2010. Shade level effects on yield and chemical composition of the leaf essential oil of *Pothomorphe umbellata* (L.) Miquel. Sci. Agric. 67, 414–418.

Menut, C., Lamaty, G., Zollo, P.-H.A., Kuiate, J.R., Bessière, J.-M., 1992. Aromatic plants of tropical Central Africa. IX. Chemical composition of flower essential oils of *Tithonia diversifolia* (Hemsl.) A. Gray from Cameroon. J. Essent. Oil Res. 4, 651–653.

Miranda, M.A.F.M., Varela, R.M., Torres, A., Molinillo, J.M.G., Gualtieri, S.C.J., Macías, F.A., 2015. Phytotoxins from *Tithonia diversifolia*. J. Nat. Prod. 78, 1083–1092.

Moronkola, D.O., Ogunwande, I.A., Walker, T.M., Setzer, W.N., Oyewole, I.O., 2007. Identification of the main volatile compounds in the leaf and flower of *Tithonia diversifolia* (Hemsl) Gray. J. Nat. Med. 61, 63–66.

Muoghalu, J.I., 2008. Growth, reproduction and resource allocation of *Tithonia diversifolia* and *Tithonia rotundifolia*. Weed Res. 48, 157–162.

Muoghalu, J.I., Chuba, D.K., 2005. Seed germination and reproductive strategies of *Tithonia diversifolia* (Hemsl.) Gray and *Tithonia rotundifolia* (P.M.) Blake. Appl. Ecol. Environ. Res. 3, 39–46.

Nehme, C.J., de Moraes, P.L.R., Tininis, A.G., Cavalheiro, A.J., 2008. Intraspecific variability of flavonoid glycosides and styrylpyrones from leaves of *Cryptocarya mandioccana* Meisner (Lauraceae). Biochem. Syst. Ecol. 36, 602–611.

Nishida, N., Tamotsu, S., Nagata, N., Saito, C., Sakai, A., 2005. Allelopathic effects of volatile monoterpenoids produced by *Salvia leucophylla*: inhibition of cell proliferation and DNA synthesis in the root apical meristem of *Brassica campestris* seedlings. J. Chem. Ecol. 31, 1187–1203.

Orsomando, G., Agostinelli, S., Bramucci, M., Cappellacci, L., Damiano, S., Lupidi, G., Maggi, F., Ngahang Kamte, S.L., Biapa Nya, P.C., Papa, F., Petrelli, D., Quassinti, L., Sorci, L., Vitali, L.A., Petrelli, R., 2016. Mexican sunflower (*Tithonia diversifolia*, Asteraceae) volatile oil as a selective inhibitor of *Staphylococcus aureus* nicotinate mononucleotide adenylyltransferase (NadD). Ind. Crops Prod. 85, 181–189.

Pavarini, D.P., Pavarini, S.P., Niehues, M., Lopes, N.P., 2012. Exogenous influences on plant secondary metabolite levels. Anim. Feed Sci. Technol. 176, 5–16.

Rahimmalek, M., Tabatabaei, B.E.S., Etemadi, N., Goli, S.A.H., Arzani, A., Zeinali, H., 2009. Essential oil variation among and within six *Achillea* species transferred from different ecological regions in Iran to the field conditions. Ind. Crops Prod. 29, 348–355.

Ramakrishna, A., Ravishankar, G.A., 2011. Influence of abiotic stress signals on secondary metabolites in plants. Plant Signal. Behav. 6, 1720–1731.

Rosenfeld, H.J., Aaby, K., Lea, P., 2002. Influence of temperature and plant density on sensory quality and volatile terpenoids of carrot (*Daucus carota* L.) root. J. Sci. Food Agric. 82, 1384–1390.

Sampaio, B.L., Edrada-Ebel, R., Da Costa, F.B., 2016. Effect of the environment on the secondary metabolic profile of *Tithonia diversifolia*: a model for environmental metabolomics of plants. Sci. Rep. 6, 29265.

Sánchez-Mendoza, M.E., Reyes-Ramírez, A., Cruz Antonio, L., Martínez Jiménez, L., Rodríguez-Silverio, J., Arrieta, J., 2011. Bioassay-guided isolation of an anti-ulcer compound, tagitinin C, from *Tithonia diversifolia*: role of nitric oxide, prostaglandins and sulfhydryls. Molecules 16, 665–674.

Satyal, P., Crouch, R.A., Monzote, L., Cos, P., Awadh Ali, N.A., Alhaj, M.A., Setzer, W.N., 2016. The chemical diversity of *Lantana camara*: analyses of essential oil samples from Cuba, Nepal, and Yemen. Chem. Biodivers. 13, 336–342.

Scrivanti, L.R., Zunino, M.P., Zygadlo, J.A., 2003. *Tagetes minuta* and *Schinus areira* essential oils as allelopathic agents. Biochem. Syst. Ecol. 31, 563–572.

Silva, M.A.S., Ming, L.C., Pereira, A.M.S., Bertoni, B.W., Batistini, A.P., Pereira, P.S., 2006. Phytochemical and genetic variability of *Casearia sylvestris* Sw. from São Paulo State Atlantic Forest and Cerrado populations. Rev. Bras. Plantas Med. 8, 159–166.

Silva, F.C.d., 2009. Manual de análises químicas de solos, plantas e fertilizantes, 2nd ed. EMBRAPA, Brasília.

Singh, H.P., Batish, D.R., Kaur, S., Arora, K., Kohli, R.K., 2006. α-Pinene inhibits growth and induces oxidative stress in roots. Ann. Bot. 98, 1261–1269.

Stashenko, E.E., Martínez, J.R., Ruíz, C.a., Arias, G., Durán, C., Salgar, W., Cala, M., 2010. *Lippia origanoides* chemotype differentiation based on essential oil GC–MS and principal component analysis. J. Sep. Sci. 33, 93–103.

Sun, W., Chen, G., Wang, S.,2007. Characteristics of *Tithonia diversifolia*: an alien invasive plant in Yunnan, south-west China. In: 3rd Global Botanic Gardens Congress. Botanic Gardens Conservation International, Wuhan, pp. 1–7.

Telascrea, M., de Araújo, C.C., Marques, M.O.M., Facanali, R., de Moraes, P.L.R., Cavalheiro, A.J., 2007. Essential oil from leaves of *Cryptocarya mandioccana* Meisner (Lauraceae): composition and intraspecific chemical variability. Biochem. Syst. Ecol. 35, 222–232.

Telci, I., Bayram, E., Yılmaz, G., Avcı, B., 2006. Variability in essential oil composition of Turkish basils (*Ocimum basilicum* L.). Biochem. Syst. Ecol. 34, 489–497.

Tongma, S., Kobayashi, K., Usui, K., 2001. Allelopathic activity of Mexican sunflower [*Tithonia diversifolia* (Hemsl.) A. Gray] in soil under natural field conditions and different moisture conditions. Weed Biol. Manag. 1, 115–119.

Tongma, S., Kobayashi, K., Usui, K., 1998. Allelopathic activity of Mexican sunflower (*Tithonia diversifolia*) in soil. Weed Sci. 46, 432–437.

Vallat, A., Gu, H., Dorn, S., 2005. How rainfall, relative humidity and temperature influence volatile emissions from apple trees *in situ*. Phytochemistry 66, 1540–1550.

Vilela, E.C., Duarte, A.R., Naves, R.V., Santos, S.C., Seraphin, J.C., Ferri, P.H., 2013. Spatial chemometric analyses of essential oil variability in *Eugenia dysenterica*. J. Braz. Chem. Soc. 24, 873–879.

Vokou, D., Kokkini, S., Bessiere, J.-M., 1993. Geographic variation of Greek oregano (*Origanum vulgare* ssp. *hirtum*) essential oils. Biochem. Syst. Ecol. 21, 287–295.

Ward, J.H., 1963. Hierarchical grouping to optimize an objective function. J. Am. Stat. Assoc. 58, 236–244.

Zhao, G.-J., Xi, Z.-X., Chen, W.-S., Li, X., Sun, L., Sun, L.-N., 2012. Chemical constituents from *Tithonia diversifolia* and their chemotaxonomic significance. Biochem. Syst. Ecol. 44, 250–254.