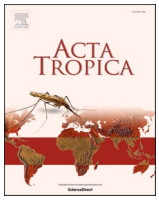
Acta Tropica 256 (2024) 107274

Contents lists available at ScienceDirect

Acta Tropica

journal homepage: www.elsevier.com/locate/actatropica



Seasonal and circadian evaluation of *Ageratum conyzoides* essential oil and its nematicidal activity against *Caenorhabditis elegans*

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ARTICLE INFO

Keywords:
precocene I
Circadian rhythm
Seasonality
Nematicidal effect

ABSTRACT

The aim of this study was to evaluate the circadian and seasonal variation of *Ageratum conyzoides* essential oil (EO) and its nematicidal effect on the free-living nematode *Caenorhabditis elegans* as a model for parasitic helminths. For the seasonal study, the plants were collected from January to December 2022, at 6 a.m., and to assess the circadian rhythm, the plants were collected in April (rainy season) and October (dry season), at 6, 9, 12 a.m. and 3 and 6 p.m. The fresh plants were then subjected to hydrodistillation, and their chemical composition was analyzed using gas chromatography coupled with mass spectrometry (GC-MS). The motility test with *C. elegans* was carried out. The primary constituent of the oils was precocene I (65.97 to 78.42 %, respectively), followed by *E*-caryophyllene (6.04 to 12.16 %), comprising an average of 79.87 % of the composition throughout the year. The average yields of EOs were slightly higher in the rainy season, at 0.68 %, compared to the dry season, at 0.62 %. High light hours in the rainy season (12 a.m., 0.96 %) and in the dry season (9 a.m., 0.88 %) seem to contribute to higher daily oil yields. It was observed that the variation between the main constituents of *A. conyzoides* occurs in inverse proportion when analyzing the main classes of compounds present in the oils: chromenes (CH) and sesquiterpene hydrocarbons (SH). And that the month of March had the highest content of *E*-caryophyllene (12.16 %) when compared to the other months of the year. On the other hand, January and December had the lowest levels of precocene I (65.97 and 66.85 %). The IC_{50} of the EO of *A. conyzoides* varied according to the month and time of collection. The EO obtained in January was the most effective against *C. elegans*, with an IC_{50} of 0.01 mg/mL. Thus, *A. conyzoides* EO could be an alternative for nematode control, exhibiting greater efficacy if extracted during specific seasonal periods.

1. Introduction

Parasitism by nematodes has caused great damage to the world economy, causing serious problems in agriculture, livestock and human health (Machado et al., 2015). Losses are estimated at between 10 to 15 % of all agricultural production worldwide, equivalent to more than US$ 100 billion per year (Tejo et al., 2020). In animals, gastrointestinal nematodes cause subclinical infections and serious clinical infections, leading to production losses and, in extreme cases, animal mortality (Miró et al., 2022).

In recent years, the control of these nematodes has been carried out mainly through chemical control with synthetic anthelmintic drugs. Although this method is widely used, it has disadvantages such as environmental toxicity, and excessive use leads to the rapid selection of resistant parasites, thereby diminishing the effectiveness of the treatment (Gilleard et al., 2021).

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https://doi.org/10.1016/j.actatropica.2024.107274
Received 8 April 2024; Received in revised form 20 May 2024; Accepted 25 May 2024
Available online 27 May 2024

The search for new control methods has therefore been intense with a view to controlling these nematodes. With this in mind, essential oils (EOs) extracted from plants can be used to obtain new bioactives with nematicidal action, due to the chemical diversity found. *Ageratum conyzoides* (Asteraceae, *Ageratum* genus) is an annual herb, fast-growing in tropical regions and with a long history of use in traditional medicine in Brazil and other countries, a variety of secondary metabolites belonging to different chemical classes, such as flavonoids, alkaloids, chromenes, terpenoids, coumarins and sterols have been isolated and characterized. These secondary metabolites are known to have various medicinal properties, including antidiabetic, antimicrobial, anti-inflammatory, antioxidant, anticancer, wound healing, antifungal, antiehrlichia, carrapaticidal and other properties (Chahal et al., 2021; do Rosário et al., 2023, 2019; Paul et al., 2022) including the nematicidal activity of its botanical extract (Baral et al., 2022; Mamman, 2023; Poné et al., 2011). The (EO) of plants of the genus *Ageratum* has also demonstrated its nematicidal effect, as is the case with *Ageratum fastigiatum* (Borges et al., 2018).

However, the chemical composition of the EO is quite variable, with quantitative and qualitative differences, as the plant does not behave in the same way all year round. It changes and, as a result, the concentration of the active ingredients can vary throughout the seasons. External factors such as temperature, rainfall, wind, soil, latitude, altitude and season significantly affect the production and yield of these compounds (Neves et al., 2021).

According to Chagas et al. (2011) harvesting is one of the main aspects to be observed in the production of quality medicinal plants. It should be carried out to obtain the maximum possible yield of EO. Understanding the species' behavior about the climatic effects of the region will imply both maximizing the yield of its EO and knowledge of the variation in its chemical composition and biological activity. This study aimed to evaluate the seasonal and circadian variation of *A. conyzoides* EO and its nematicidal effect on the free-living nematode *Caenorhabditis elegans* as a model for parasitic helminths.

2. Materials and methods

2.1. Plant material and oil extraction

The species *A. conyzoides* was collected in the city of São Luís, MA, Brazil, coordinates: 2°38′07" S and 44°19′16" W. The exsiccate was deposited in the MAR Herbarium, Federal University of Maranhão (UFMA), São Luís, MA, Brazil, under number 9099. For the seasonal study, the plants were collected from January to December 2022, at 6 a.m., and to assess the circadian rhythm, the collections were made in April (rainy season) and October (dry season), at 6, 9, 12 a.m., 3 and 6 p.m. The experiments were carried out in triplicate. The plants were collected in accordance with Brazilian laws on the protection of biodiversity (SisGen n° A82AB5C). The EO were extracted from the fresh aerial parts (300 g) by hydrodistillation (3 h) using a Clevenger-type apparatus, then the oil was dried over anhydrous sodium sulphate and its yield was calculated (v/w).

2.2. Oil-composition analysis

The chemical composition was analyzed by Gas Chromatography coupled to Mass Spectrometry (GC-MS) on a Shimadzu QP 2010 ultra system with the injection of 1 μL of 3:500 oil in hexane solution (Auto injector AOC-20i), an Rtx-5MS silica capillary column (Restek, USA) 30 m long x 0.25 mm internal diameter coated with 5 %-diphenyl/95 %-dimethyl-polysiloxane (0.25 μm film thickness) was used. The temperature of the GC oven was programmed from 60°C to 240°C (10 min) at 3°C/min, the temperatures of the injector (split 1:20), transfer line and ionization chamber were 250, 250 and 200°C, respectively. Helium was used as the carrier gas at a flow rate of 1mL/min. Mass spectra were obtained by electron impact at 70 eV with automatic scans in the range of 35 to 400 daltons at 0.30 scans/s. Identification of the components was based on the linear retention time and index (C8-C40 n-alkane series), interpretation and comparison of the mass spectra obtained with the Adams (2007) and Mondello (2011).

2.3. Maintenance of caenorhabditis elegans

Adults of *C. elegans*, susceptible (wild strain, Bristol N2), were maintained in a nematode growth medium (NGM) and fed with the *Escherichia coli* NA22 bacteria (Sigma-Aldrich, USA), at 22°C. Young L4 worms and adult nematodes aged four to five days old were isolated from the culture plates, selected in an inverted optical microscope (Carl Zeiss, Oberkochen, Germany), and used for the *C. elegans* motility test.

2.4. Caenorhabditis elegans motility test

The motility test was performed according to Katiki et al. (2013). *A. conyzoides* of EO (20 mg/mL) was diluted in 3 % Tween 80 for preparing the stock solution. Subsequently, 100 μL of the stock solution was transferred to a 96-well plate and added more 100 μL saline solution (M-9) containing KH_2PO_4 (3 g), Na_2HPO_4 (6 g), NaCl (5 g), $MgSO_4$ (1.0 mL), and H_2O (1.0 L). A balanced saline solution (M-9) was used for the preparation of the different treatments for subsequent addition nematodes. About 50 adult nematodes were added in 200 μL of oil and M-9 solution. The concentration of oil different, varied from 0.001 to 10.0 mg/mL, with three replicates for each concentration. The solutions were incubated for 24 h, at 24 °C. After this period, the number of live and dead nematodes was evaluated using an inverted microscope (Carl Zeiss). Nematodes without movement during five seconds of observation were considered dead. As negative and positive controls were used M9 solution and 3 % Tween 80 and ivermectin (0.00024 mg/mL), respectively.

2.5. Statistical analysis

The obtained data were subjected to nonlinear regression to get 50 % inhibitory concentration (IC_{50}), using the GraphPad Prism 8.0 software (GraphPad Inc., San Diego, USA). Significant differences between the strains were considered ($p<0.05$ ANOVA).

Multivariate statistical analysis was performed according to Cruz et al. (2023). Statistical significance was assessed using the Tukey test ($p<0.05$). GraphPad Prism software, version 8.0, was used to calculate Pearson's correlation coefficients (r). Principal component analysis (PCA) was applied to verify the inter-relationship in the oil components (>2 %).

3. Results and discussion

3.1. Yield and composition of oils in the seasonal and circadian study

The yield of *A. conyzoides* EOs during the seasonal study are shown in Fig. 1. The evaluation of the seasonal influence on the production and composition of the EO of *A. conyzoides* during one year (from January to December 2022) was monitored by the climatic parameters: sunshine (mar:78.8 - sep: 241.1 h), temperature (mar: 26.39 - oct: 27.8°C), relative humidity (oct: 81.61 - mar: 92.58 %) and rainfall (oct: 0 - mar: 641.1 mm). The rainy season ran from January to May and the dry season from June to December, according to the rainfall (Fig. 1).

Oil yields were highest in March (1.21 %) and June (1.03 %) and lowest in October (0.58 %) and November (0.52 %). The EO yield showed no statistical difference between the dry season (0.72 ± 0.2 %) and the rainy season (0.87 ± 0.22 %) according to Student's t-test (Fig. 2). According to Person's correlation coefficient (r), the EO yield showed a significant moderate correlation ($p<0.05$) with rainfall (r = 0.673) and a strong significant correlation with temperature (r = -0.720).

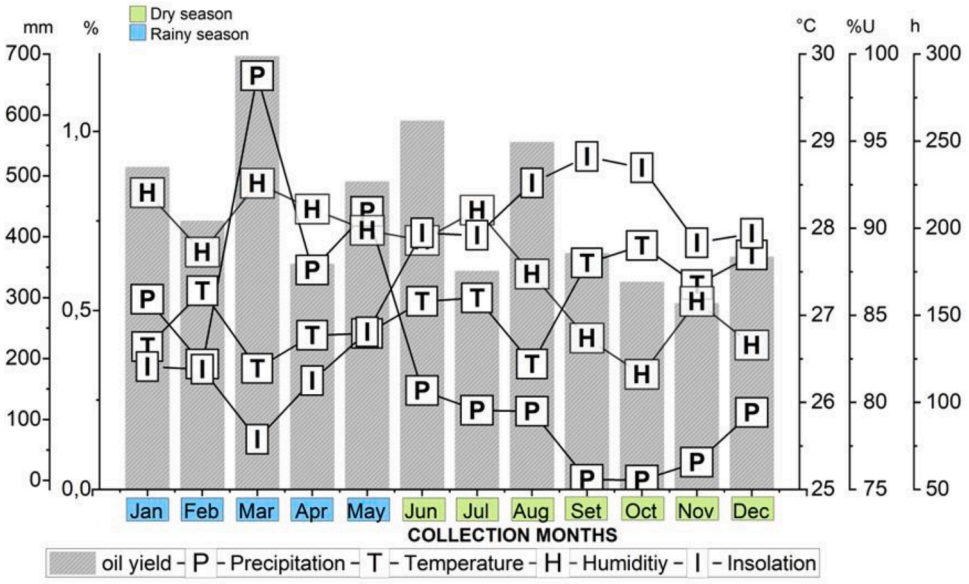

Fig.. 1. Relationship between essential oil yield and seasonal parameters.

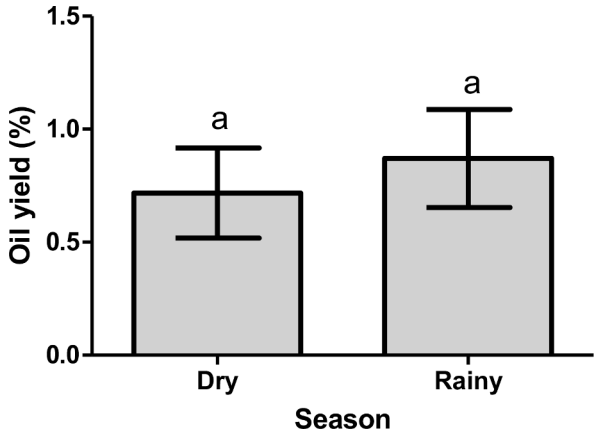

Fig.. 2. Essential oil yield during dry and rainy seasons. Values with the same letters (a) in the bars do not differ statistically in the Tukey test ($p>0.05$).

Sixty-two constituents were identified and quantified by GC-MS, representing 99.07 % of the total oils (representative ion-chromatograms and main compounds mass spectra are shown in Supplementary Material). The main constituent of the oils was precocene I (65.97 to 78.42 %), followed by *E*-caryophyllene (6.04 to 12.16 %), comprising an average value of 79.87 % of the oils' composition. The oil extracted in April had the highest levels of precocene I (78.42 %) and the lowest levels of *E*-caryophyllene (6.04 %). March had the highest *E*-caryophyllene content (12.16 %) when compared to the other months of the year. On the other hand, January and December had the lowest levels of precocene I (65.97 and 66.85 %).

The major constituents (*E*-caryophyllene and precocene I) showed a negligible and insignificant correlation with the climatic parameters. Thus, the climatic parameters (temperature, rainfall and humidity) may correlate with the yield of *A. conyzoides* EO, but they do not correlate with the majority constituents and the classes of compounds identified (Monoterpene hydrocarbons, oxygenated monoterpene, sesquiterpene hydrocarbons, oxygenated sesquiterpene, chromenes, fatty acids and derivatives), as shown in Table 1.

The bioactives precocene I and precocene II have been isolated from plants throughout the family Asteraceae (Bede and Tobe, 2000). Indeed, these compounds belong to the acetate pathway. However,

Table. 1
Correlation between chemical composition, oil yield and seasonal parameters.

	Precipitation	Temperature	Humiditiy	Insolation
Yield	0.673*	0.720*	0.558	-0.433
***E*-Caryophyllene**	0.303	-0.128	-0.197	-0.078
Precocene I	-0.106	-0.068	0.198	0.146

* $p<0.05$

E-caryophyllene is a sesquiterpene belonging to mevalonate/Methylerythritol phosphate pathways. So, these compounds belong to different biosynthesis pathway, which are few correlated (Dewick, 2009).

From the average content and standard deviation of the majority constituents (*E*-caryophyllene and precocene I) present in the EO of the species *A. conyzoides* in the dry (D) and rainy (R) seasons (Fig. 3), *E*-caryophyllene (R = 9.24 ± 2.78 %; D = 8.93 ± 1.70 %) and precocene I (R =70.08 ± 4.81 %; D = 71.33 ± 3.03 %) show no statistical difference during the dry and rainy seasons.

Principal component analysis (PCA) used the constituents and months of study as the data matrix, explaining 86.40 % of the values obtained (Fig. 4). PC1 explains 66.40 % of the data and shows a negative correlation with precocene I (λ=-0.427), a positive correlation with *E*-caryophyllene (λ=0.274), *α*-Humulene (λ=0.477), Germacrene D (λ=0.484), trans-Muurola-4(14),5-diene (λ=0.456) and β-Sesquiphellandrene (λ=0.269). PC2 explains 20.00 % of the data and shows a positive correlation with Germacrene D (λ=0.103), Precocene I (λ=0.202), trans-Muurola-4(14),5-diene (λ=0.267) and *β*-Sesquiphellandrene (λ=0.661) and a negative correlation with *E*-caryophyllene (λ=-0.637) and *α*-Humulene (λ=-0.186).

The PCA showed a homogeneous distribution between the samples of *A. conyzoides* EO during the dry and rainy periods, with no difference between the samples in the two periods. Environmental factors only had an influence on the EO yield of the species. Studies on the seasonal chemical variability of *A. conyzoides* EOs have not been found in the literature. The PCA showed that there was no separation between the oil samples from the dry and rainy periods, see Fig. 4 above.

The variation between the main constituents is inversely proportional when analyzing the main classes of compounds present in the oils: chromenes (CH) and sesquiterpene hydrocarbons (SH). January saw a variation of CH= 67.12 % and SH = 20.06 %, i.e. the month with the lowest variation in CH and the highest in SH, compared to the other

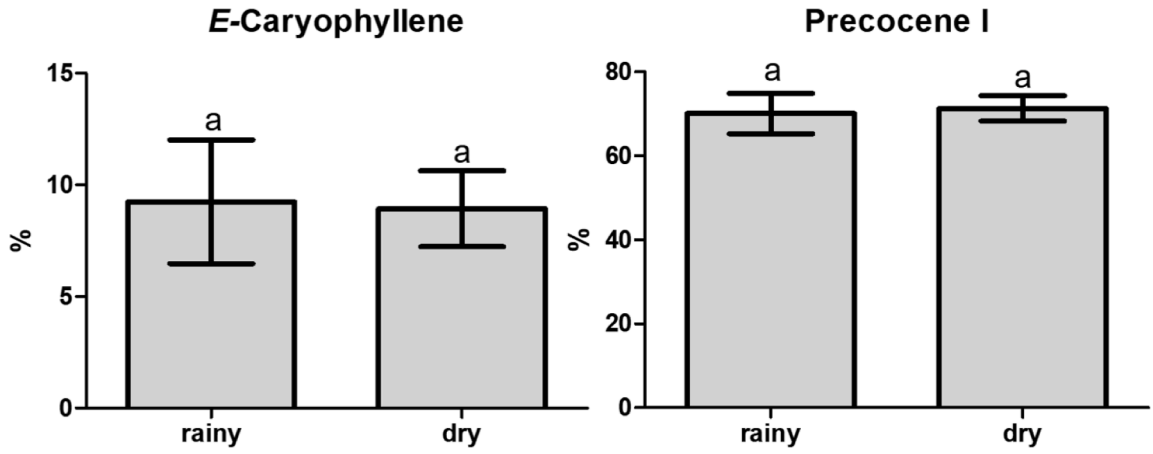

Fig. 3. Main essential oil component during rainy and dry seasons. Values with the same letters (a) in the bars do not differ statistically in the Tukey test (p>0.05).

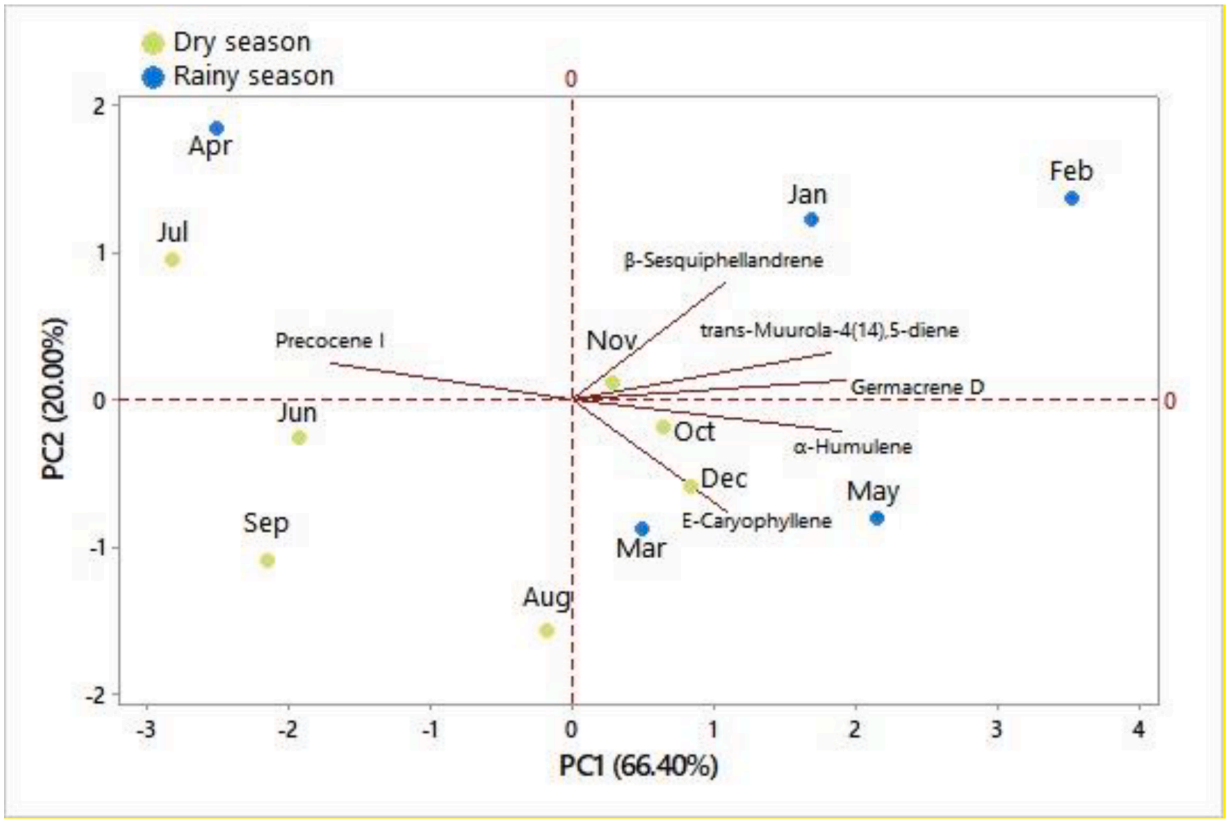

Fig. 4. Principal components analysis (PCA) of the *Ageratum conyzoides* oils from seasonal study, based on their primary constituents.

months of the year. In April, June and July, CH = 79.44, 75.68 and 76.36 % and SH = 13.82, 16.34 and 13.83 % were the months with the greatest variation in CH and the least in SH. Around 0.6 % of the EO composition of the seasonal rhythm was also made up of fatty acids and their derivatives (Table 2).

The yield and composition of *A. conyzoides* EOs during the circadian study are described in Table 3. Average oil yields were slightly higher in the rainy season, at 0.68 %, compared to the dry season, at 0.62 %. High light hours in the rainy season (12 a.m., 0.96 %) and the dry season (9 a. m., 0.88 %) seem to contribute to higher daily oil yields. Sixty-eight constituents were identified and quantified by GC-MS, representing 97.95 % of the total oils.

The highest levels of precocene I were observed at 6 a.m. (78.39 %) and 6 p.m. (74.47 %) in the rainy season, while *E*-caryophyllene showed the lowest levels (6.04 and 7.5 %) at the same times of day and seasonal period. In the dry season, the highest levels of precocene I were found in the morning (6 a.m., 68.98 % and 9 a.m., 64.42 %). It's worth noting that in the rainy season, the highest *E*-caryophyllene levels were found in the periods with the most sunlight (9 a.m., 12.79 % and 12 a.m., 10.92 %). In the dry season, the yields of this constituent showed increasing levels over the circadian rhythm (6 a.m., 8.78 %; 9 a.m., 12.51 %; 12 a. m., 14.1 %; 3 p.m., 14.64 % and 6 p.m.15.38 %).

The daily variation in the rainy season for chromenes (CH) and sesquiterpenes hydrocarbons (SH) was CH, 73.89 %, and SH, 19.38 %, respectively. For the dry period, the variation was CH, 65.21 %, and SH, 24.74 %, respectively, with more significant values. Around 0.6 % of the composition of seasonal oils is also made up of fatty acids and their derivatives. The dry season showed an average value for fatty acids and

Table. 2
Seasonal variation of the *Ageratum conyzoides* essential oil.

Yield (%)			0.9	0.75	1.21	0.63	0.86	1.03	0.61	0.97	0.66	0.58	0.52	0.65
Constituent (%)	RI_C	RI_L	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
3*Z*-Hexenol	850	850		0.05	0.15	0.08	0.06	0.06	0.02	0.03	0.02	0.05	0.02	0.03
Camphene	949	946[a]	0.01		0.02				0		0.09			
Sabinene	973	972[b]	0.02	0.03	0.02	0.04	0.02			0.03	0.02		0.02	0.03
β-Pinene	978	978[b]	0	0		0.01	0	0.01	0.02	0	0.01	0.02	0	
Borneol	1166	1173[b]			0.05		0.03	0.01			0.03	0.01		
Naphthalene	1181	1186			0.18		0.01							
α-Terpineol	1191	1195[b]			0.03					0.01	0.03			
Isobornyl formate	1228	1235[a]	0.02	0.04	0.54		0.28	0.16	0.04	0.13	0.62	0.07	0.05	0.09
Lavandulyl acetate	1280	1289			0.02			0.01		0.01	0.03			
Bornyl acetate	1286	1287[a]	0.18	0.18	1.42	0.03	0.83	0.69	0.28	0.68	1.9	0.5	0.26	0.37
Eugenol	1358	1357	0.04				0.02	0.1		0.01	0.02		0.03	0.04
Neryl acetate	1365	1362	0.01	0.01		0.01	0.04	0.08	0.07	0.08	0.14	0.12	0.03	0.02
Linalool isobutyrate	1370	1374[a]					0.01	0.03	0.01	0.01	0.02			
α-Copaene	1377	1375[b]	0.04	0.07	0.06	0.01	0.09	0.03	0.01	0.05	0.04	0.05	0.04	0.05
β-Bourbonene	1386	1384	0.04	0.05	0.05	0.01	0.06	0.03	0.03	0.05	0.04	0.1	0.05	0.06
β-Cubebene	1391	1392[b]	0.63	0.98	0.81	0.21	1.17	0.4	0.27	0.75	0.54	0.77	0.56	0.63
β-Elemene	1393	1389[a]	0.4	0.37	0.22	0.16		0.23	0.16	0.26	0.23	0.32	0.33	0.35
E-Caryophyllene	1423	1424[b]	6.54	10.37	12.16	6.04	11.09	8.24	6.17	11.06	8.71	8.78	8.5	11.06
β-Copaene	1430	1433	0.02	0.02			0.02			0.01		0.02	0.01	
α-*trans*-Bergamotene	1437	1432[a]	0.07	0.09	0.09	0.03	0.07	0.05	0.09	0.03	0.03	0.13	0.05	0.08
(*Z*)-β-Farnesene	1444	1445	0.39	0.2	0.24	0.11	0.18	0.2	0.34	0.11	0.09	0.49	0.18	0.21
Prezizaene	1453	1450[a]	0.04		0.08				0.03	0.06	0.05			0.07
α-Humulene	1455	1452[a]	2.17	2.49	1.9	1.2	2.6	1.42	1.08	2.06	1.47	2.13	1.94	1.9
Precocene I	1477	1464[b]	65.97	68.36	68.8	78.42	68.87	74.4	74.97	71.54	73.13	68.98	69.45	66.85
trans-Cadina-1(6),4-diene	1480	1476[a]		0.1	0.08	0.06	0.11			0.09	0.08	0.06		0.08
γ-Muurolene	1483	1476	0.08	0.08	0.06	0.04	0.07	0.06	0.04	0.06	0.05	0.08	0.07	0.06
Germacrene D	1486	1484[a]	3.13	3.51	2.1	1.66	3.35	1.75	1.29	2.17	1.5	2.49	2.51	2.45
β- *trans*-Bergamotene	1488	1480[d]	0.4		0.39	0.29		0.27	0.59			0.68	0.42	0.37
trans-Muurola-4(14),5-diene	1496	1493[a]	1.37	2.02	0.83	0.64	1.25	0.67	0.54	0.88	0.57	1.15	0.97	0.87
Germacrene A	1496	1509[a]	0.37											
γ-Amorphene	1497	1495[a]									0.27		0.29	
epi-Cubebol	1498	1488				0.34	0.37	0.23	0.26			0.42		
Byciclogermacrene	1500	1500 [a]	0.91	0.04	0.57	0.06	0.12	0.67	0.46	0.08	0.44	0.09	0.72	1.29
α-Muurolene	1503	1500[a]	0.26	0.28	0.16	0.13	0.22	0.12	0.11	0.16	0.12	0.22	0.20	0.17
α-Bulnesene	1509	1504		0.36	0.24	0.17	0.35	0.28	0.15	0.26	0.18	0.27	0.31	0.26
β-Bisabolene	1511	1508[b]	0.11	0.18	0.16	0.14	0.09		0.13	0.07	0.06	0.11	0.12	0.16
β-Curcumene	1514	1512	0.02	0.02	0.02	0.01	0.02	0.02	0.03	0.01	0.01	0.03	0.03	0.02
δ-Cadinene	1518	1522[a]	0.5	0.34	0.21	0.24	0.31	0.22	0.23	0.27	0.21	0.36	0.35	0.3
β-Sesquiphellandrene	1526	1523[b]	2.51	3.23	2.37	2.59	2	1.58	2.07	1.37	1.15	1.92	2.12	2.36
trans-Cadina-1,4-diene	1535	1528[a]	0.06	0.04	0.02	0.02	0.04	0.03	0.01	0.02	0.02	0.03	0.03	0.02
cis-Sesquisabinene *Hydrate*	1545	1541	0.11	0.04		0.04	0.03							0.05
E-Nerolidol	1565	1561[a]	0.94	0.45	0.86	0.66	0.52	1.09	1.22	0.6	0.89	1.03	1.11	1.02
3-hexenyl benzoate	1572	1569	0.01					0.02	0.03			0.02		
trans-Sesquisabinene *Hydrate*	1581	1577[a]	0.42	0.06	0.11	0.11	0.07	0.12	0.31			0.22	0.15	
Caryophyllene oxide	1584	1592[a]	1.03	0.45	0.61	0.56	0.49	0.69	1.25	0.87		1.04	0.9	1.42
Guaiol	1590	1603[b]	0.3			0.17	0.15		0.12					
Epoxide humulene II	1610	1613[b]	0.19	0.06		0.05	0.06	0.05	0.09	0.08	0.09	0.12	0.09	0.08
1,10-di-epi-Cubenol	1615	1612					0.04		0.07	0.03		0.16		0.16
γ-Eudesmol	1627	1630		0.01		0.03	0.01		0.02					
1-*epi*-Cubenol	1630	1625	0.33	0.2	0.1	0.15	0.21	0.17	0.13	0.17	0.13		0.2	
Caryophylla-4(12),8(13)-dien-5-α-ol	1634	1640	0.39	0.07	0.08	0.16	0.11	0.16	0.35	0.21	0.25	0.22	0.24	0.26
Caryophylla-4(12),8(13)-dien-5- β-ol	1638	1639	0.78	0.2	0.23	0.4	0.26	0.4	0.74	0.5	0.63	0.5	0.51	0.62
α-Muurolol (=Torreyol)	1643	1644[a]								0.22	0.19	0.26	0.31	
Cubenol	1644	1636	0.4	0.29	0.19	0.22	0.27	0.24	0.22					0.23
Desmethoxy encecalin	1650	1646[a]	1.19	0.38	0.33	0.39	0.42	0.55	0.7	0.32	0.52	0.67	0.73	0.54
α-Cadinol	1656	1653[a]	0.52			0.29	0.33	0.36		0.34				
14-hidroxy-9-*epi*-(*E*)-Caryophyllene	1673	1668[a]					0.08				0.17		0.04	
Andro encecalinol	1679	1675[a]	0.08	0.73	0.49	0.63	0.43	0.73	0.69	0.55	0.56	0.56	0.76	0.76
α-Bisabolol	1685	1682	0.63	0.04	0.04	0.04	0.03	0.06	0.07		0.04	0.06	0.07	0.06
Germacra-4(15),5,10(14)-trien-1-α-ol	1688	1684		0.08		0.13		0.16	0.21					
Hexahydrofarnesyl-acetone	1845	1843	0.02	0.01				0.01	0.04	0.01	0.02	0.05	0.02	0.02
Fitol	2112	2116	0.21	0.27	0.32	0.27	0.29	0.29	0.56	0.32	0.17	0.67	0.62	0.35
Monoterpene hydrocarbons (MH) (%)			0.03	0.03	0.04	0.05	0.02	0.01	0.02	0.03	0.12	0.02	0.02	0.03
Oxygenated monoterpene (OM) (%)			0.25	0.23	2.24	0.04	1.22	1.08	0.4	0.93	2.79	0.7	0.37	0.52
Sesquiterpene hydrocarbons (SH) (%)			20.06	24.84	22.85	13.82	23.31	16.34	13.83	19.88	15.86	20.3	20.01	23.14
Oxygenated sesquiterpene (OS) (%)			6.08	2.29	3.04	4.04	3.52	4.6	6.42	3.56	3.45	5.25	5.06	5.16
Chromenes (CH) (%)			67.12	69.47	69.62	79.44	69.72	75.68	76.36	72.41	74.21	70.21	70.94	68.15
Fatty acids and derivatives (%)			0.24	0.38	1.28	0.36	0.6	0.66	0.69	0.5	0.97	0.94	0.75	0.55
Total (%)			93.78	97.24	99.07	97.75	98.45	98.37	97.72	97.31	97.4	97.42	97.15	97.55

RI_C = Calculated Retention Index (Rxi-5ms column); RI_L = Literature Retention Index
[a] = Adams, 2007;
[b] = Mondello, 2011,
[d] =Terpenoids Library Website.

Table. 3
Circadian rhythm of the *Ageratum conyzoides* essential oil chemical composition during the rainy and dry seasons.

Yield (%)			0.63	0.67	0.96	0.66	0.49	0.58	0.88	0.61	0.57	0.47
Constituent (%)	RI_C	RI_L	April (rainy season)					October (dry season)				
			6 a.m.	9 a.m.	12 a.m.	3 p.m.	6 p.m.	6 a.m.	9 a.m.	12 a.m.	3 p.m.	6 p.m.
3Z-Hexenol	850	850[a]	0.08	0.21	0.11	0.09	0.06	0.05		0.03		
2-E-hexenol	860	854[a]	0.01	0.02					0.06			
α-Pinene	934	932[a]		0.01					0.06		0.02	0.01
Camphene	949	946[a]		0.05	0.04		0.01		0.38	0.01	0.03	0.13
Sabinene	973	972[b]	0.04	0.03	0.02	0.03	0.03		0.03			0.03
β-Pinene	978	978[b]	0.01		0.01			0.02	0.05	0.02	0.01	0.02
δ-2-Carene	1003	1000[b]		0.06	0.03				0.2			0.1
α-Phellandrene	1006	1007[b]		0.02					0.02			0.01
Limonene	1028	1024[a]		0.03	0.02				0.19		0.1	0.11
4,8-dimethyl-, (E)-Nona-1,3,7-triene	1116	1113[a]		0.01					0.1	0.02		0.1
Borneol	1166	1173[b]		0.02	0.02		0.01	0.01	0.03	0.01	0.03	0.02
Isobornyl formate	1228	1235[a]		0.1	0.16	0.03	0.05	0.07	0.33	0.19	0.27	0.33
Bornyl acetate	1286	1287[a]	0.03	0.42	0.74	0.16	0.28	0.5	1.17	0.85	0.96	1.13
α-Cubebene	1351	1351		0.06					0.03	0.02	0.28	0.02
Neryl acetate	1365	1362	0.01	0.06	0.06	0.03	0.05	0.12		0.07	0.05	0.06
α-Copaene	1377	1375[b]	0.01		0.05	0.02	0.03	0.05	0.11	0.09		0.1
β-Bourbonene	1386	1384	0.01	0.05	0.05	0.02	0.04	0.1	0.15	0.1		0.1
β-Cubebene	1391	1392[b]	0.21	0.79	0.62	0.31	0.58	0.77	1.14	0.98	0.87	1.04
β-Elemene	1393	1389[a]	0.16	0.36	0.31	0.25	0.28	0.32	0.44	0.34	0.22	0.25
2-*epi*-β-Funebrene	1415	1411[a]		0.02				0.02				
E-Caryophyllene	1423	1424[b]	6.04	12.79	10.92	7.5	8.33	8.78	12.51	14.1	14.64	15.38
β-Copaene	1430	1433		0.01		0.01	0.01	0.02		0.02		0.02
α-*trans*-Bergamotene	1437	1432[a]	0.03	0.09	0.13	0.06	0.03	0.13	0.13	0.13	0.11	0.13
epi-β-Santalene	1437	1427[a]				0.05						
Coumarin	1442	1434[a]		0.05	0.03	0.03		0.08				0.03
(*Z*)-β-Farnesene	1444	1445	0.11	0.21	0.37	0.17		0.49	0.35	0.35		0.35
Prezizaene	1453	1450[a]		0.06	0.06	0.04			0.08	0.08		0.07
α-Humulene	1455	1452[a]	1.2	2.18	1.45	1.67	1.87	2.13	2.24	2.17	2.10	2.08
Precocene I	1477	1464[b]	78.39	66.6	71.31	72.76	74.47	68.98	64.42	62.2	62.7	62.46
trans-Cadina-1(6),4-diene	1480	1475[a]	0.06	0.08	0.07	0.07	0.09	0.06	0.07			0.07
γ-Muurolene	1483	1476	0.04	0.07	0.06	0.05	0.06	0.08	2.51	0.07	0.05	0.06
Germacrene D	1486	1484	1.66	2.59	1.47	2.43	2.29	2.49	0.42	2.4	2.49	2.1
β- *trans*-Bergamotene	1488	1480[d]	0.29	0.34	0.59	0.35		0.68	0.97	0.52	0.67	0.51
trans-Muurola-4(14),5-diene	1496	1493[a]	0.64	1.02	0.57	0.68	0.95	1.15		1.24	0.96	0.84
Germacrene A	1496	1509[a]				0.22						
cis-Cadina-1,4-diene	1497	1531			0.13				0.29			
epi-Cubebol	1498	1488	0.34	0.27		0.24	0.4	0.42			0.17	0.29
Byciclogermacrene	1500	1500[a]	0.06	0.98	2.09	1.2	0.1	0.09	0.85	0.25	0.34	0.13
α-Muurolene	1503	1500[a]	0.13	0.18	0.1	0.13	0.18	0.22	0.18	0.19	0.21	0.17
α-Bulnesene	1509	1504	0.17	0.24	0.16		0.27	0.27	0.24	0.22		0.23
β-Bisabolene	1511	1508[b]	0.14	0.18	0.19	0.17	0.09	0.11	0.13	0.14		0.12
β-Curcumene	1514	1512	0.01	0.02	0.03	0.02	0.01	0.03	0.02	0.03		0.02
δ-Cadinene	1518	1522[a]	0.24	0.26		0.22	0.29	0.36	0.27	0.26	0.25	0.24
β- Sesquiphellandrene	1526	1523[b]	2.59	2.85	2.43	2.56	1.85	1.92	2.04	2.46	2.59	2.14
trans-Cadina-1,4-diene	1535	1528[a]	0.02	0.03	0.02	0.03	0.03	0.03	0.02	0.03	0.01	0.02
cis-Sesquisabinene *Hydrate* (IPP vs. OH)	1545	1541	0.04	0.03		0.04	0.03			0.06	0.05	0.06
epi-Longipinanol	1555	1562[a]		0.04		0.84						
E-Nerolidol	1565	1561[a]	0.66	0.6	0.65		0.54	1.03	0.92	1.03	0.93	0.87
Espathulenol	1579	1575[b]		0.03		0.04						
trans- Sesquisabinene *Hydrate* (IPP vs. OH)	1581	1577[a]	0.11	0.07	0.17	0.11		0.22		0.12	0.15	
Caryophyllene oxide	1584	1592	0.56	0.5		0.63	0.74	1.04	1.03	1.6	1.68	1.73
Carotol	1590	1597[a]		0.12								
Viridiflorol	1590	1594[b]		0.01		0.04			0.03			
Guaiol	1590	1603[b]	0.17			0.12	0.18				0.10	0.13
Humulene epoxide II	1610	1613[b]	0.05	0.04		0.06	0.07	0.12	0.08	0.12	0.15	0.11
1-*epi*-Cubenol	1630	1625	0.15	0.13	0.06	0.14	0.2		0.13	0.16	0.13	0.11
Caryophyll -4(12),8 (13) - dien-5-α-ol	1634	1640	0.16	0.12	0.14	0.13	0.2	0.22	0.19		0.15	0.33
Caryophyll -4(12),8 (13) - dien-5-β-ol	1638	1639	0.4	0.25	0.28	0.35	0.45	0.5	0.44	0.37	0.64	0.82
α-Muurolol (=Torreyol)	1643	1644[a]						0.26	0.2	0.86	0.35	
Cubenol	1644	1636	0.22	0.19	0.12	0.26	0.26			0.24	0.17	0.13
Desmethoxy encecalin	1650	1646[a]	0.39	0.65	0.41	0.63	0.36	0.67	0.52	0.5	0.48	0.3
α-Cadinol	1656	1653[a]	0.29			0.38				0.36	0.19	
Andro encecalinol	1679	1675[a]	0.63	0.62	0.51	1.27	0.43	0.56	0.5	0.77	0.65	0.6
α-Bisabolol	1685	1682	0.04	0.04	0.05	0.06		0.06	0.05	0.05	0.05	0.04
Germacra-4(15),5,10 (14) - trien-1-α-ol	1688	1684	0.13	0.1		0.14	0.19				0.17	0.23
2*Z*,6*Z*-Farnesol	1690	1718[a]			0.01	0.05			0.02		0.01	0.02
Hexahydrofarnesyl-acetone	1845	1843					0.01	0.05	0.02	0.04	0.03	0.04
Fitol	2112	2116	0.27	0.32	0.21	0.3	0.26	0.67	0.3	0.39	0.3	0.23
Monoterpene hydrocarbons (%)			0.05	0.18	0.12	0.03	0.04	0.02	0.96	0.03	0.16	0.42
Oxygenated monoterpenes (%)			0.04	0.5	0.82	0.19	0.35	0.7	1.31	0.99	1.04	1.27
Sesquiterpene hydrocarbons (%)			13.82	25.46	21.87	18.23	17.55	20.3	25.23	26.21	25.79	26.19
Oxygenated sesquiterpene (%)			4.04	2.43	1.48	3.69	3.37	5.25	3.25	5	5.12	4.91

(*continued on next page*)

Table. 3 (*continued*)

Yield (%)			0.63	0.67	0.96	0.66	0.49	0.58	0.88	0.61	0.57	0.47
Constituent (%)	RI_C	RI_L	April (rainy season)					October (dry season)				
Chromenes (%)			79.44	67.87	72.23	74.69	75.26	70.21	65.44	63.47	63.59	63.36
Fatty acids and derivatives (%)			0.36	0.85	0.51	0.46	0.39	0.97	0.86	0.69	0.57	0.79
Total (%)			97.95	97.29	97.03	97.29	96.96	96.13	97.05	96.39	96.27	96.94

RI_C = Calculated Retention Index (Rxi-5ms column); RI_L = Literature Retention Index
[a] = Adams, 2007
[b] = Mondello, 2011
[d] =Terpenoids Library Website.

their derivatives of around 0.8 %, compared to the rainy season, with 0.5 %.

Apart from the common constituents identified in the seasonal and circadian cycle oils, the presence of other terpenoids and fatty acid derivatives, in small quantities, was observed in one or other of these oils. In the seasonal study they were Naphthalene, α-Terpineol, Lavandulyl acetate, eugenol, Linalool isobutyrate, γ-Amorphene, γ-Eudesmol and 14-hydroxy-9-epi-*(E)*-caryophyllene (Table 2). In contrast, the circadian study included 2-*E*-hexenol, α-Pinene, δ-2-Carene, α-Phellandrene, Limonene, 4,8-dimethyl-, *(E)*-Nona-1,3,7-triene, α-Cubebene, 2-epi-β-Funebrene, epi-β-Santalene, Coumarin, *trans*-Muurola-4(14),5-diene, *cis*-Cadin-1,4-diene, epi-Longipinanol, Spathulenol, Carotol, Viridiflorol and 2*Z*,6*Z*-Farnesol (Table 3).

Fresh plant samples were used in both the seasonal and circadian studies. Although dried material is more chemically stable due to the interruption of metabolic processes that occur even after the plant has been collected, the use of fresh material is indispensable in this species for the production and detection of some specific components.

Stimuli arising from the environment in which the plant is found can redirect the metabolic pathway, causing the biosynthesis of different compounds. This is due to temporal and spatial variations in the total content, as well as the relative proportions of secondary metabolites at different levels (seasonal and daily; intra-plant, inter- and intra-specific) since secondary metabolites represent a chemical interface between plants and the surrounding environment. Therefore, their synthesis is often affected by environmental conditions (Gobbo-Neto and Lopes, 2007).

Variations in precocene I and *E*-caryophyllene in the EO of *A. conyzoides* have been reported in different regions. In Ghana, Mensah et al. (1993) found 80.29 % precocene I and 7.04 % *E*-caryophyllene. Menut et al. (1993) in Cameroon found 81 % precocene I in the same oil. A study carried out in Portugal reported levels of 34.4 % precocene I and 24.6 % *E*-caryophyllene (Martins et al., 2005). Kouame et al. (2018) found variations of these same constituents in oils extracted from different parts of the plant in Cote d'Ivoire: precocene I (flower: 58.8 %, stem: 76.5 %, *E*-caryophyllene (flower: 15.2 %, stem: 8.1 %).

Differences in the levels of these same constituents (precocene I-74.30 % and *E*-caryophyllene -14.23 %) were also reported in Brazil, in a study carried out by de De Melo et al. (2011) in São Paulo. These differences, as mentioned above, can be explained by environmental factors during plant growth (Pintong et al., 2020).

3.2. Anthelmintic activity

The inhibitory concentration (IC_{50}) of the *A. conyzoides* OE exhibited variation according to the collection month. The oil of *A. conyzoides* obtenaid in January showed lower inhibitory concentration among the oils tested, with an IC_{50} value of IC_{50} 0.01 mg/mL (IC 95 %= 0.043-0.105) for Bristol N2 strain of *C. elegans,* followed by April IC_{50} of 0.07 mg/mL (Table 4). Control positive (ivermectin) tested in 0.00024 mg/mL exhibited 100 % efficiency against the N2 strain.

However, for April in 9 a.m. the IC_{50} concentration increased. The oil obtained in the dry season at October 6 p.m. and 9 a.m. were less efficient against *C. elegans.* Thus, it was observed that the EO obtained during the rainy season was more efficient (Fig. 5).

Table. 4
Inhibitory concentration (IC_{50}) of the essential oils of *Ageratum conyzoides* collected at rainy and dry season against *Caenorhabditis elegans* adults.

Collection/Seasonal	Month	IC_{50} (mg/mL)	CI95 %	R^2
Rainy	January	0.01	0.012-0.017	0.96
	April	0.07	0.043-0.105	0.82
Dry	July	0.09	0.078-0.116	0.95
	October	0.15	0.107-0.214	0.87

The biological activity of EOs can be related to their majority and minority compounds individually or through their synergism, as the relationship between chemical compounds is very complex and can affect the characteristics of the EO as well as its biological action (Jiang et al., 2009).

In addition, differences in the composition of the EO and its biological activity may be due to different stages of development or variations in the plant's growing conditions, or as a result of structural or physiological modifications to the plant caused by specific environmental factors, causing significant changes in its content (Gong et al., 2014). With regard to the particularities of the oil, some research into its composition has shown that even intra-specific genetic variations in the plant species can alter the content of the active ingredient present (Nascimento et al., 2007; Oka et al., 2000).

Mamman (2023) demonstrated that the active compounds of

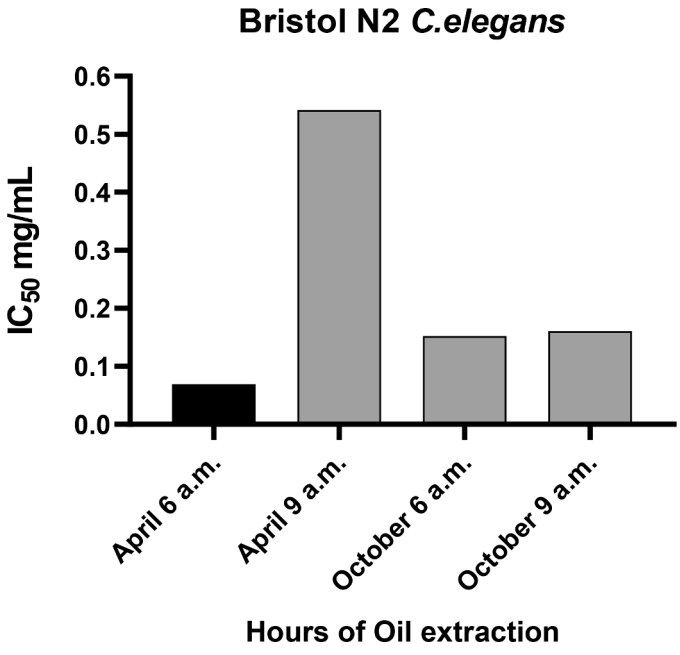

Fig. 5. Inhibitory concentration (IC_{50}) of the essential oils of *Ageratum conyzoides* collected at different times of the day against *Caenorhabditis elegans* adults. Horizontal lines on the same level or grouping the same bars do not significantly differ by the confidential interval 95 %.

A. conyzoides have anthelmintic properties. However, studies have already described that *E*-caryophyllene, present in reasonable (second majority compound) amounts in *A. conyzoides* EO, is effective against various helminths, such as *Trichostrongylus* spp., *Haemonchus* spp., *Syphacia obvelata* and *Aspiculuris tetraptera* (Camurça-Vasconcelos et al., 2008, 2007). The lipophilic properties of EO terpenoids could potentially be related to their damaging effect on nematode cell membranes (Echeverrigaray et al., 2010; (Ntalli et al., 2010; Costa et al., 2016; D'Addabbo et al., 2020). Additionally, De Melo et al. (2011), evaluated the EO of *A. conyzoides* and its main isolated compounds (precocene I and *E*-caryophyllene) against *Schistosoma mansoni* (trematodes). The results indicated that the EO demonstrated greater activity than its isolated main components.

Furthermore, chromenes, which comprise around 78 % of the constituents of the EOs in this study, stand out for being known as a broad group of natural products with notable relevance for their chemical and biological activities (Costa et al., 2016). They are compounds with redox action in various types of oxidative biological processes, being links in the electron transport chain in the metabolic pathway. The biocidal activity of chromenes is enhanced by the presence of a double bond system that favors processes involving electron transfer (Pinto and Castro, 2009).

Thus, the chemical structure of phenols plays an important role in their ability to eliminate free radicals and related reactive species formed in many physiological processes and the antioxidant activity exerted by these compounds may also be reasonably involved in their toxic effects on nematodes (D'Addabbo and Avato, 2021). Thus, the EO of *A. conyzoides* could be an alternative for nematode control, with greater efficiency if this product is extracted in certain seasonal periods, such as the month of January, which showed the best nematicidal activity and an optimum yield of EO was obtained, given that discrete variations were observed throughout the year in relation to this parameter, and we even propose that this would be the most appropriate time for harvesting.

In addition, *C. elegans*, is an excellent alternative parasite for identifying several new natural products activities that may offer additional treatments against nematode infections that constitute a serious problem for human health and agricultural production (Mathew et al., 2016). These results support the hypothesis that the combination of *A. conyzoides* EO constituents may be useful as a nematicidal product against various helminths.

4. Conclusion

As reported, an excellent yield of EOs extracted from *A. conyzoides* was observed throughout the year, slightly higher in the rainy season, as well as a slight chemical variation in its compounds over the months, with precocene I and caryophyllene being the majority components. As for the efficacy of *A. conyzoides* EO against *C. elegans*, it was observed that the EO obtained in January was the most effective against the nematode, with an IC_{50} of 0.01 mg/mL. Thus, the EO extracted from *A. conyzoides* could be an alternative to commercially available synthetic nematicides. It represents an important source of bioproducts, since its excellent yield associated with a discreet chemical variation in its compounds, observed in seasonal and circadian evaluations, makes its economic exploitation viable.

Financial support

This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Finance Code 001, PROCAD-AM [88887.472618/2019-00 (CQR)] and Fundação de Amparo à Pesquisa e ao Desenvolvimento Científico e Tecnológico do Maranhão – Brasil (FAPEMA) – BPD - [12125/22] (CJRMR)].

Availability of data and materials

All data generated or analyzed during this study are included in this published article in the main manuscript and additional file.

Ethics approval

Not applicable.

CRediT authorship contribution statement

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.actatropica.2024.107274.

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