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Essential oil *Ageratum conyzoides* chemotypes and anti-tick activities

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ABSTRACT

Rhipicephalus (Boophilus) microplus represents a significant obstacle to animal productivity in tropical and subtropical areas, leading to considerable economic losses for the dairy and meat production industries. Essential oils (EO) extracted from *Ageratum conyzoides* are known to cause death and induce morphogenetic abnormalities in several insect species. This plant, however, presents morphological flower variations, which range from white to purple, associated to different chemotypes. In this context, this study aimed to conduct a novel assessment on the effects of EO extracted from two *A. conyzoides* chemotypes in the control of the bovine tick *R. microplus*. The primary constituents of the oil obtained from white flower samples (WFs) were precocene I (80.4%) and (E)-caryophyllene (14.8%), while purple flower oil samples (PFs) contained predominantly β-acoradiene (12.9%), γ-amorphene (12.3%), α-pinene (9.9%),

bicyclogermacrene (8.9%), α-santalene (8.7%), and andro encecalinol (5.6%). Interestingly, only the EO chemotype from *A. conyzoides* PFs displayed acaricidal activity towards *R. microplus* larvae, with an LC_{50} of 1.49 mg/mL.

Keywords:

Natural compound, Asteraceae, Acaricide, tick.

1. Introduction

Exotic plant species have invaded ecosystems worldwide, leading to extensive problems which have, in turn, led to the recognition that the occupation of natural communities comprises a significant threat to the planet's biodiversity. The herb *Ageratum conyzoides* is a good example of this. Belongs to the Asteraceae family and comprise about thirty species allocated in the Eupatoriae tribe, is an annual and invasive aromatic herb that can reach up to 1.0 m in various soil types and is widespread along roadsides and wastelands in Africa, Asia, and South America. This plant presents a peculiar male goat odor, hence the common name in Brazil of "Catinga-de-bode" (goat smell), as well as “Mentrasto” and “Erva-de-São-João”. It exhibits morphological flower variations, which range from white to purple, with a close terminal-type inflorescence arrangement (Okunade, 2002; Pintong et al., 2020; Kong et al., 2004; Singh et al., 2011; Yadav et al., 2019).

Although it is harmful to crops, invading cultivated fields and interfering with the natural composition of several ecosystems, at the same time this species is also employed as a medicinal plant in various countries, exhibiting diverse biological activities. *Ageratum conyzoides* contains different secondary metabolites, *i.e.,* flavonoids, chromenes, benzofurans, and terpenoids, with some presenting allelopathic activities to other surrounding species (Kong et al., 2004; Singh et al., 2011; Yadav et al., 2019).

Ageratum conyzoides is noteworthy for its widespread medicinal use in Brazil and other countries, displaying several therapeutic properties, such as antioxidant, antibacterial,

antimicrobial, anticancer, and acaricide activities, among others (Cox, 1994; Paul et al., 2022). Ethanolic *A. conyzoides* extracts have also been proven as presenting anti-tick activity against the larvae of sensitive and multidrug-resistant *Rhipicephalus* (*Boophilus*) *microplus* strains (Kamboj and Saluja, 2008; Yadav et al., 2019; Widyawati et al., 2021; Kumar et al., 2022).

Rhipicephalus microplus (Canestrini 1887) is the most important cattle tick species worldwide (Marques et al., 2020). This parasite represents a significant obstacle to animal productivity in tropical and subtropical areas, leading to considerable economic losses for the milk and meat production industries (Burrow et al., 2019; Grisi et al., 2014). Estimates suggest that the total economic loss attributable to *R. microplus* infestations in the Brazilian cattle herd is approximately US$ 3.2 million per year (Grisi et al., 2014; Calvano et al., 2019). Adopting strategic measures to reduce *R. microplus* populations in livestock translates into financial returns by increasing productivity. Moreover, tick resistance to synthetic acaricides has increased in the face of the indiscriminate use of many different products (Higa et al., 2016). Thus, the search for natural plant-based products has become essential as a sustainable strategy aiming at tick control.

Essential oils (EO) constitute secondary plant metabolite blends, usually extracted by steam distillation. These metabolites comprise low molecular weight volatile molecules containing terpenoid and phenylpropanoid/benzenoid-type constituents (Bakkali et al., 2008). The insecticidal or acaricidal efficacies of various EO have been well-documented against many pests attributed to significant EO components or their synergistic effects that lead to cellular accumulation and toxic insect nervous system effects. Simultaneously, the hydrophobic nature of these oils may exert a mechanical effect on parasites, disrupting cuticular waxes and blocking spiracles, leading to death by water stress or suffocation (Ellse and Wall, 2014). An example of this is terpinen-4-ol, an oxygenated monoterpene found in high amounts in the EO extracted

from *Melaleuca alternifolia* (Myrtaceae), which act by inhibiting the acetylcholinesterase enzyme in arthropods (Mills et al., 2010).

The EO extracted from *A. conyzoides* can cause death and induce morphogenetic abnormalities in several arthropod species. This has been attributed to the presence of the chromenes precocene I and precocene II, which cause premature metamorphosis in several arthropods, leading to sterile adults (Gbolade et al., 1999; Pintong et al., 2020). In addition to the presence of precocene I and II, β-caryophyllene, caryophyllene oxide, β-cubebene, β-bisabolene, germacrene D, α-santalene, α-humulene, and α-pinene have also been reported as the main constituents of EO extracted from *A. conyzoides* (Mensah et al., 1993; Martins et al., 2005; Zoghbi et al., 2007; Kamboj and Saluja, 2008; Usman et al., 2013; Kouame et al., 2017).

Essential oil composition is quite variable, presenting quantitative and qualitative differences due to environmental alterations, such as temperature, relative humidity, sun exposure, rainfall, and wind regimes, associated to the development of different plant chemotypes (Gobbo-Neto and Lopes, 2007). These variations are responsible for differences in biological properties, *i.e*, anticancer, antifungal and antioxidant activities (de Castro et al., 2019; Fernandes et al., 2021). Although *A. conyzoides* extracts have been reported as displaying as anti-tick proprieties, EO with different chemotypes have not yet been tested. In this context, this study aimed to analyze the effect of EO from two *A. conyzoides* chemotypes against the cattle tick *R. microplus.*

2. Materials and Methods

2.1. Plant material and oil extraction

The aerial parts of two samples of *A. conyzoides*, presenting white (WFs) and purple (PFs) flowers, were collected in the city of São Luís, MA, Brazil, at 2°38'07" S and 44°19'16" W. The botanist Eduardo Bezerra Almeida Junior identified the white flower sample (Ws), which was

deposited in the Herbarium of the Federal University of Maranhão under number 9099. The botanist Ana Maria Maciel Leite identified the purple flower sample (Ps), which was deposited in the Herbarium of the State University of Maranhão under number 6235. The plants were collected in agreement with Brazilian laws concerning the protection of biodiversity (SisGen n° A82AB5C). The essential oils 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 sulfate, and its yield was calculated (v/w) (Lima et al., 2021).

2.2. Oil-composition analysis

The oils analysis was performed on a GCMS-QP2010 Ultra system (Shimadzu Corporation, Tokyo, Japan) equipped with the GCMS-Solution software containing the Adams (2007) and Mondello (2011) libraries. A Rxi-5ms (30m x 0.25mm; 0.25µm film thickness) silica capillary column (Restek Corporation, Bellefonte, PA, USA) was used. The conditions of analysis were: injector temperature of 250°C; oven temperature programming of 60-240°C (3°C/min); helium as the carrier gas, adjusted to a linear velocity of 36.5 cm/s (1.0 mL/min); split mode injection for 1.0 µL of the sample (oil 6.0 µL : hexane 500 µL); split ratio 1:20; ionization by electronic impact at 70 eV; ionization source and transfer line temperatures of 200 and 250°C, respectively. The mass spectra were obtained by automatically scanning every 0.3 s, with mass fragments in the 35-400 m/z. The retention index was calculated for all oil components using a homologous series of C8-C40 n-alkanes (Sigma-Aldrich, USA), according to the linear equation of Van den Dool and Dec. Kratz (1963). The oil components were identified by comparing their retention indices and mass spectra (molecular mass and fragmentation pattern) with those existing in the GCMS-Solution system libraries. The quantitative data regarding the oil constituents were obtained using the GC 2010 Series coupled to a flame ionization detector (FID), operated under similar conditions to the GC-MS system.

2.3. Tick collection and rearing

Engorged *R. microplus* susceptible to cypermethrin (Santa Rita strain) (≥ 4.5 mm) were collected from artificially infested calves, without recent contact with acaricides. Engorged *R. microplus* collected were washed with water, dried with a paper towel and kept under controlled laboratory conditions (27 ± 2˚C and relative humidity ≥ 80%) for 15 days until the eggs were laid. After the egg hatching, larvae 14 to 21 days aged were used for the larval immersion test (LIT). The UFMA ethics committee approved this study under number 23.115.008186/2017-18.

2.4. Larval immersion test

The *R. microplus* larvae immersion test was performed according to Klafke et al. (2006). Briefly, different concentrations (500-5000 μg/mL) of the essential oils were prepared, diluted in 1.0% ethanol and 0.02% Triton X-100 solution, which served as a negative control of the test. Then, 1 mL of the different concentrations was transferred to microtubes, and approximately 500 larvae were added to each tube with the treatment and control solution for 10 min. The larvae were then dried, and about 100 larvae were transferred to a filter paper envelope (8.5 cm x 7.5 cm), with subsequent sealing, and then were kept in an incubator at 27 ± 1°C and relative humidity 85% for 24 h. After this time, alive and dead larvae were counted. The experiment was performed with four replicates for each treatment. The data were initially transformed to Log(X), normalized and the nonlinear regression was used to calculate LC_{50}. Significant differences in the average efficiency of each oil (WFs and PFs) were considered when there was no overlap between the 95% confidence limits of the LC_{50} values (Roditakis et al. 2005).

3. Results and discussion

3.1. Oil-composition analysis

The yields and composition of EO extracted from *A. conyzoides* WFs and PFs are depicted in Table 1. Fifty-seven constituents were identified and quantified by GC-MS and GC-FID, averaging 99.6% of the total oil content. The primary WFs constituents were precocene I (80.4%) and (E)-caryophyllene (14.8%), while PFs EO contained mainly β-acoradiene (12.9%), γ-amorphene (12.3%), α-pinene (9.9%), bicyclogermacrene (8.9%), α-santalene (8.7%), and andro encecalinol (5.6%). The variations between the main EO constituents is also highlighted when analyzing their main secondary metabolite classes, namely (1) chromenes (80.4%) and sesquiterpene hydrocarbons (18.2%) in WFs and (2) sesquiterpene hydrocarbons (59.2%), monoterpene hydrocarbons (26.6%), and oxygenated sesquiterpenes (11.7%) in PFs. These findings indicate that at least two different *A. conyzoides* chemical types occur in São Luis, MA, Brazil.

Table 1 Oils composition from two *Ageratum conyzoides* chemotypes.

Yields (%)			0.7	0.4
Constituents (%)	RI_C	RI_L	WFs	PFs
α-Thujene	927	924[a]		0.2
α Pinene	936	932[a]		**9.9**
Camphene	952	946[a]		0.2
Sabinene	975	972[b]		0.5
β-Pinene	980	978[b]		0.2
Myrcene	991	988[a]		3.0
δ-2-Carene	1000	1000[b]		0.5
α-Phellandrene	1008	1007[b]		0.3
α-Terpinene	1019	1018[b]		0.5
p-Cymene	1027	1025[b]		0.3
Limonene	1032	1030		3.1
(*E*)-β-Ocimene	1049	1046		1.8
γ-Terpinene	1062	1058[b]		3.5
Terpinolene	1088	1086[a]		2.6
δ-Terpineol	1177	1170[b]		0.4
Borneol	1179	1173[b]		0.1
Terpinen-4-ol	1186	1180[b]		0.2
Isobornyl formate	1236	1235[a]	0.2	
Bornyl acetate	1290	1287[a]	0.9	1.1
δ-Elemene	1339	1335[a]		0.2
α-Copaene	1385	1375[b]		0.8
β-Cubebene	1395	1392[b]	0,3	
β-Elemene	1397	1389[a]		3.3
α-Bergamotene	1420	1416[b]		0.5
α-Santalene	1428	1418[b]		**8.7**
(*E*)-Caryophyllene	1432	1424[b]	**14.8**	
γ-Elemene	1433	1432[b]		4.1
α-trans-Bergamotene	1440	1432[a]		0.3

α-Humulene	1455	1452[a]	0.3	
β-Santalene	1457	1457[a]		3.0
β-Acoradiene	1471	1469[a]		**12.9**
Precocene I	1473	1464[b]	**80.4**	
9-epi-(*E*)-Caryophyllene	1474	1464[a]		0.2
Amorpha-4,7(11)-diene	1487	1479[a]		1.6
γ-Amorphene	1496	1495[a]	1.3	**12.3**
Viridiflorene	1503	1496[a]		0.5
α-Muurolene	1505	1500[a]	0.5	
Bicyclogermacrene	1510	1500[a]		**8.9**
β-Bisabolene	1513	1508[b]		0.4
Sesquicineole	1522	1515[a]		0.1
δ-Cadinene	1527	1522[a]		0.7
β-Sesquiphellandrene	1530	1523[b]	1.0	0.1
(*E*)-γ-Bisabolene	1534	1529[a]		0.7
Occidentalol	1555	1550[a]		0.1
(*E*)-Nerolidol	1564	1561[a]	0.2	0.2
Spathulenol	1575	1575[b]		0.2
Gleenol	1590	1586[a]		0.2
Caryophyllene oxide	1597	1592[a]		0.1
Viridiflorol	1600	1594[b]		0.2
Guaiol	1610	1603[b]		0.4
Humulene epoxide II	1614	1613[b]		0.8
allo-Aromadendrene epoxide	1649	1644[b]		0.4
α-Muurolol	1655	1651[b]		1.2
Intermedeol	1669	1665[a]		1.4
14-hydroxy-9-*epi*-(*E*)-Caryophyllene	1672	1668[a]		0.5
Andro encecalinol	1680	1675[a]		**5.6**
(Z)-α-Santalol acetate	1780	1778[b]		0.3
Monoterpene hydrocarbons			-	26.6
Oxygenated monoterpenes			1.1	1.8
Sesquiterpene hydrocarbons			18.2	59.2
Oxygenated sesquiterpenes			0.2	11.7
Chromenes			80.4	-
Total (%)			99.9	99.3

RI_C = Calculated Retention Index (Rxi-5ms column); RI_L = Literature Retention Index ([a] = Adams, 2007; [b] = Mondello, 2011); WFs = white flower sample; PFs = purple flower sample; Bold = Main constituents (above 5%).

Two other chemical *A. conyzoides* types have been identified in previous studies concerning specimens from the state of Pará, Brazil, one comprising predominantly precocene I and (*E*)-caryophyllene, and another with α-pinene, α-santalene, germacrene D, α-humulene, and β-bisabolene as the primary constituents (Zoghbi et al., 2007). *A. conyzoides* produces a volatile oil with a strong odor and varying chemical compositions in different countries (Kouame et al., 2017).

Essential *A. conyzoides* oils containing predominantly precocene I and (E)-caryophyllene have been reported in different regions, *i.e.*, Ghana (Mensah et al., 1993), Cameroon (Menut et al., 1993), Portugal (Martins et al., 2005), São Paulo, Brazil (de Melo et al., 2011), Nigeria (Usman et al., 2013), Ivory Coast (Kouame et al., 2017), Thailand (Pintong et al., 2020). These differences, as mentioned previously, can be explained by environmental factors during plant growth (Pintong et al., 2020).

3.2 *Anti-tick effects*

The novelty of this study concerns the acaricidal action of the EO chemotype extracted from *A. conyzoides* PFs against *R. microplus* considering the larval stage of the ixodid, with a determined LC_{50} of 1.49 mg/mL (CI 95% 1,136 to 1,920 mg/mL, R^2:0.83). The acaricidal activities of α-pinene and β-pinene have been proven as 100% effective against *R. microplus* larvae (Prates et al., 1993). The EO of the precocene-rich chemotype, however, did not exhibit considerable activity against *R. microplus* in the present study (Figure 1).

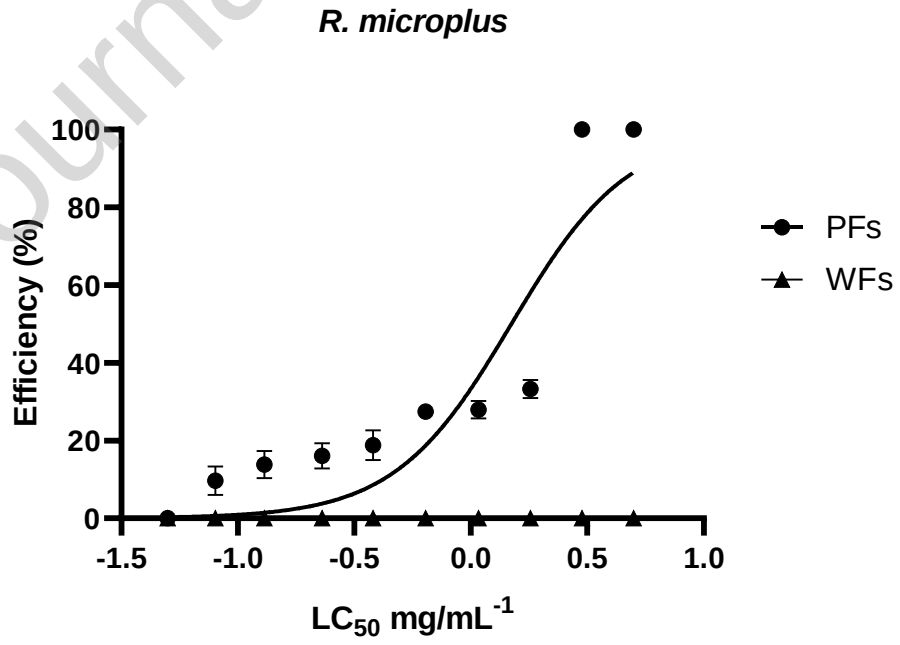

Figure 1: Lethal concentrations (LC_{50}) of essential oils extracted from *Ageratum conyzoides* white flower sample (WFs) and purple flower sample (PFs) against *Rhipicephalus microplus* larvae.

The *in vitro* efficacies of ethanolic extracts obtained from *A. conyzoides* have been reported as 53.77 and 77-90% against pyrethroid-resistant and multi-acaricide-resistant *R. microplus* strains, respectively (Kumar et al., 2016). Along these same lines, Parveen et al. (2014) reported 4 0% activity of the same extract with a 60 % decrease in egg masses when applied at a 20 % concentration against *R. microplus* adults.

Studies have reported that precocene II leads to a 99.9% *R. microplus* larval mortality rate at 4.25 mg/ml (0.425%) (Ribeiro et al., 2011). Kumar et al. (2022), on the other hand, observed that higher precocene II acaricidal activity compared to precocene I, but with a weak positive correlation (r= 0.11) concerning tick mortality. However, significant differences have been noted between precocene I and II contents in EO extracted from aerial *A. conyzoides* portions from different regions (Nogueira et al., 2010).

Precocene II was not identified in the extracted EOs in the present study, probably associated to plant maturity, as precocene I, characteristic of young plants, is the biosynthetic precursor of precocene II, with is usually higher in mature plants (Siebertz et al., 1990). This may be the reason why no tick control effect was observed for the EO precocene I-rich chemotype. Another hypothesis is that the insecticidal action of chromenes (precocene I and precocene II) is due to antijouvenile hormones, leading to antigonadotropic and ovicidal effects, early metamorphosis and diapause induction in insects, and not a knock down effect as observed herein against *R. microplus* larvae (Ming, 1999).

Chromenes, sesquiterpenes and monoterpenes are known as insecticidal compounds (Kong et al., 2004). Essential oil contact with the insect thorax triggers hyperactivity, followed by hyperexcitation and a quick drop to the bottom of experimental vessels, suggesting

neurotoxicity most of the acaricides act by effecting on motor function by inhibitory effects on the activity of monoamine oxidase (MAO), most likely through the increases produced on catecholamine levels within the central nervous system (Salman et al., 2020).

Essential oils are characterized by two or three major components present at relatively high concentrations (20-70%) alongside other less abundant compounds. Major EO components generally determine biological EO properties (Bakkali et al., 2008), although the activity of these major components may also be modulated by less abundant compounds (Campos et al., 2011). The EO presenting the highest acaricidal activity in the present study is a blend containing low amounts of several compounds. Studies employing mixtures of different compound should, thus, be performed to identify the mixtures with the highest acaricidal activity.

Chemical composition varies with climate, season, geographical conditions, harvest period, and distillation technique (Knaak and Fiuza, 2010). Thus, in addition to the analyzed marker compounds, other molecules may also play an important role in tick mortality. The mechanism of action of EO in arthropods is still unknown, but studies suggest essential oils have different modes of action, producing various biological changes in target organisms. Some of the main biological effects of essential oils may be cytotoxicity, phototoxicity, mutagenicity, carcinogenicity, and neurotoxicity. Essential oils are also antimutagenic (Salman et al., 2020).

Conclusion

Based on the findings reported herein, the EO extracted from purple *A. conyzoides* flowers may be an alternative to commercially available synthetic acaricides. Further investigations concerning different *A. conyzoides* chemotypes are, however, required to determine the true potential of *A. conyzoides* in the control of cattle tick infestations.

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Data availability

All data are available as part of this article.

Declarations

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.

Conflict of interest

The authors declare no conflicts of interest in relation to this work.

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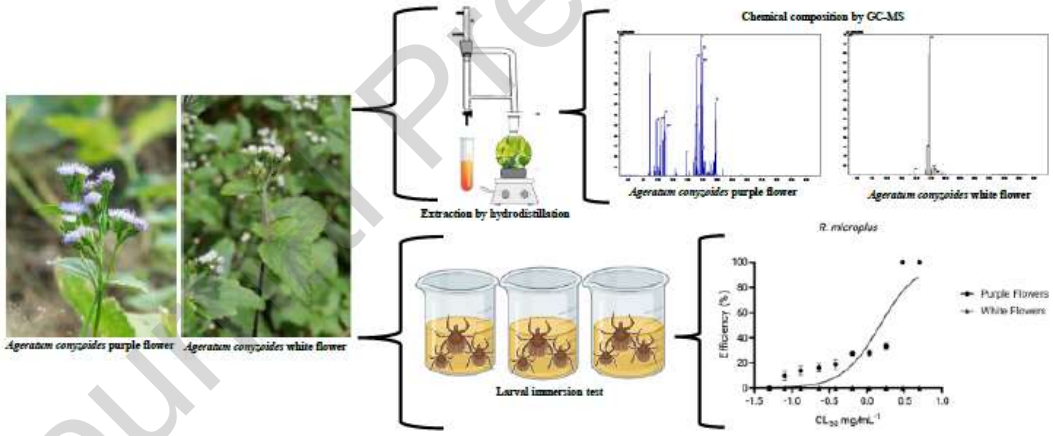
CRediT authorship contribution statement

ROSARIO CJRM: Methodology, Validation, Investigation, Formal analysis, Writing – original draft. LIMA AS, MENDONÇA CJ, ALMEIDA JÚNIOR EB, COSTA-JUNIOR LM, GOMES MN, SOARES IS: Methodology, Validation, Investigation. LIMA AS, MAIA JG: Conceptualization, Investigation, Resources, Writing – review & editing. ROCHA CQ: Methodology, Validation, Conceptualization, Investigation, Resources, Writing – original draft, Writing – review & editing, Project administration, Funding acquisition, Formal analysis.

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.

Graphical abstract



Highlights

- The essential oil of *Ageratum conyzoides* shows acaricidal activity.
- *Ageratum conyzoides* chemotypes show different results in the antiparasitic action.
- No tick control action has been observed in the EO of the chemotype rich in precocene I.