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Change in leaf anatomy, physiology, and essential oil of *Varronia curassavica* Jacq. accessions under two light conditions

[Cambio en la anatomía de la hoja, fisiología y aceite esencial de accesiones de *Varronia curassavica* Jacq. bajo dos condiciones de luz]

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Abstract: The aim of this study was to evaluate the effect of light on anatomy, physiology and essential oil content and composition of *Varronia curassavica*. They were analysed two light conditions (full sunlight and protected environment with 50% shade screen) and five accessions (VCUR-101, VCUR-102, VCUR-201, VCUR-302, VCUR-802). *V. curassavica* cultivated in full sun presented a greater development of the leaf blade and palisade parenchyma thickness for all accessions. Chlorophyll levels did not differ according to the two light environments. The leaf area was larger in the protected environment. The essential oil yield of the accessions ranged from 0.26 to 0.87 mL/plant in full sun and from 0.34 to 0.53 mL/plant in the protected environment. The composition of the essential oil was influenced by the light and the accession. All accessions presented (E)-caryophyllene and α-humulene. The influence of light on the evaluated variables is genotype dependent.

Keywords: Chemical composition; Medicinal plant; Foliar anatomy; Chlorophyll contents; Shade screen

Resumen: El objetivo de este estudio fue evaluar el efecto de la luz sobre la anatomía, fisiología, el contenido y composición de aceites esenciales de *Varronia curassavica*. Se analizaron dos condiciones de luz (pleno sol y ambiente protegido con 50% de pantalla de sombra) y cinco accesiones (VCUR-101, VCUR-102, VCUR-201, VCUR-302, VCUR-802). *V. curassavica* cultivada a pleno sol presentó mayor desarrollo del limbo foliar y espesor del parénquima en empalizada para todas las accesiones. Los niveles de clorofila no difirieron según los dos entornos de luz. El área foliar fue mayor en el ambiente protegido. El rendimiento de aceite esencial de las accesiones varió de 0,26 a 0,87 mL/planta a pleno sol y de 0,34 a 0,53 mL/planta en el ambiente protegido. La composición del aceite esencial fue influenciada por la luz y la accesión. Todas las accesiones presentaron (E)-cariofileno y α-humuleno. La influencia de la luz sobre las variables evaluadas depende del genotipo.

Palabras clave: Composición química; Planta medicinal; Anatomía foliar; Contenido de clorofila; Pantalla de sombra

INTRODUCTION

Varronia curassavica Jacq. belongs to the family Cordiaceae and is popularly known as "erva-baleeira". The species is a medicinal plant native from Brazil and can be found in the coastal strip from the states of Ceará to Rio Grande do Sul (Gasparino & Barros, 2009). It is an erect and branched shrub, with simple, leathery and aromatic leaves, and small white flowers arranged in terminal inflorescences (Brandão *et al*., 2017).

V. curassavica leaves are used in traditional medicine as antiulcer, analgesic, and anti-inflammatory. Due to its use in folk medicine, pharmaceutical companies have shown interest in using its leaves as a raw material for natural medicines (Hoeltgebaum *et al*., 2018). For instance, Acheflan®, a topical herbal medicine, was developed by Ache Laboratory using the essential oil extracted from the leaves of this species (Ventrella & Marinho, 2008; Queiroz, 2009). Other studies have revealed that the aqueous extract or the essential oil of *V. curassavica* has antifungal (Nizio *et al*., 2015), antiprotozoal (Silva *et al*., 2019) and insecticidal (Oliveira *et al*., 2019) activities. The toxicity to the *Aedes aegypti* mosquito larvae (Santos *et al*., 2006) and inhibitory activity against gram-positive bacteria were also observed (Meccia *et al*., 2009; Michielin *et al*., 2011).

Essential oils are volatile compounds, consisting mainly of monoterpenes and sesquiterpenes (Azevedo *et al*., 2016), and they are products of secondary metabolism of plants. The specialized metabolites can redirect the metabolic route, causing the biosynthesis of different compounds (David *et al*., 2006; Oliveira *et al*., 2009). These modifications are directly associated with the adaptation and survival of the species to several environments. Additionally, they maximize the yield capacity and guarantee the functioning of the process of specialized metabolites synthesis.

The major compounds observed in the essential oil of *V. curassavica* are the monoterpenes δ-3-carene, α-pinene, β-pinene, sabinene, and the sesquiterpenes aristolene, allo-aromadendrene, bicyclogermacrene, β-bourbonene, α-cadinol, (*E*)-caryophyllene, ar-curcumene, β-elemene, δ-elemene, α-humulene, γ-muurolene, β-sesquiphellandrene, shyobunol, α-turmerone, β-turmerone, and ar-turmerone (Queiroz *et al*., 2016; Marques *et al*., 2019; Silva *et al*., 2019). The sesquiterpenes α-humulene and (*E*)-caryophyllene are considered the two chemical markers of *V. curassavica* (Brandão *et*

al., 2017).

Light is one of the most important environmental factors for plant growth and development, since it is the source of energy for photosynthesis (Silvestri *et al*., 2019). In addition to the changes on leaf anatomy, light can also affect the chemical composition of the essential oil. Changes in luminosity in the cultivation environment provide adjustments in the photosynthetic apparatus of the plants, what can result in greater efficiency in the absorption and energy transfer to photosynthetic processes (Souza *et al*., 2011).

Quantitative growth analysis is a fundamental tool to evaluate plant development, being very valuable in understanding how environmental and genetic factors influence growth and production of essential oils (Azevedo *et al*., 2015). The leaf area, for example, is an important variable to evaluate. Leaf area expresses the amount of mass not transported from the leaves to other parts of the plant. This variable is especially important for species whose leaves are of economic interest, such as *V. curassavica*, which essential oil production is concentrated in the leaves (Assis *et al*., 2015).

The aim of this study was to evaluate the effect of light and comparing different accessions on the anatomy, physiology and essential oil content and composition of *Varronia curassavica*.

MATERIALS AND METHODS
Plant material
Varronia curassavica accessions were collected from the Active Germplasm Bank of medicinal and aromatic plants of the Federal University of Sergipe. The collection is located at the Research Farm "Campus Rural" of the Federal University of Sergipe, in São Cristóvão, State of Sergipe, Brazil.

The accessions of *V. curassavica* used in this study were: VCUR-101 and VCUR-102 (7-methyl-3-methylene-10-(1-propyl)-7-cyclodecen-1-one, and shyobunone IV - Group V); VCUR-201 [(*E*)-caryophyllene and/or 7-methyl-3-methylene-10-(1-propyl)-7-cyclodecen-1-one - Group IV); VCUR-302 (α-zingiberene and β-sesquiphellandrene - Group III); and VCUR-802 (turmerone and (*E*)-caryophyllene - Group I). The accessions were selected based on the study of Nizio *et al*. (2015).

Seedlings were produced by cuttings in tubes containing coconut coir and sand (2:1 v/v) as the substrate. They were kept in a protected environment with 50% shade screen with a misting irrigation system for 60 days. Afterward, plants were

transferred to 5-liter pots containing soil, sand, and bovine manure [2:1:1 (v/v/v)] as the substrate.

Experimental Design

The experiment consisted of a randomized block design in a split-plot scheme, testing two light conditions (full sunlight and protected environment with 50% shade screen) in the plots, and five *V. curassavica* accessions (VCUR-101, VCUR-102, VCUR-201, VCUR-302, VCUR-802) in the subplots, with three replications and three plants per replication. For 50% shade screen, the plants were kept in greenhouse.

At 180 days of cultivation the following variables were evaluated: leaf anatomy; a, b, and total chlorophyll contents; leaf area; leaf dry weight; and content, yield and chemical composition of the essential oil of the accessions, in two light conditions.

Anatomical and physiological analyses

The analyses were carried out in the Tissue Culture and Plant Breeding Laboratory of the Department of Agronomic Engineering, Federal University of Sergipe.

For leaf anatomy, fully expanded leaves were collected from the third leaf pair, counting from the leaf apex to the base. The leaves were fixed in FAA 70 (formaldehyde, acetic acid, 70% ethyl alcohol) (Johansen, 1940) then, they were cross-sectioned into fragments of approximately 2 cm^2, considering the region of the central vein of the leaves. Cross-sections were performed in three leaves/ treatments.

To obtain permanent slides, leaf fragments were dehydrated in an increasing ethanol series. The material was embedded in hydroxyethyl methacrylate (Jung's Historesin - Leica®), according to a modified manufacturer's protocol. Samples were sectioned at the thickness of 6 μm using a semiautomatic microtome. These cross-sections were then stained with toluidine blue and mounted in stained glass. The sections were photographed under an optical microscope equipped with a LEICA DM500 camera, visualized with a computer, using the LAS EZ® software, and analysed using the UTHSCSA-Imagetool® software (version 3.0). The following variables were measured: palisade and spongy parenchymas thicknesses (μm), leaf blade thickness (μm), central vein thickness (μm), and abaxial and adaxial epidermis thicknesses (μm).

The contents of chlorophyll a, chlorophyll b, and total chlorophyll were measured in fully expanded young leaves, from the third leaf pair, counting from the leaf apex to the base, using portable chlorophyll meter (Falker). To measure these variables, three replications were used, being three leaves per plant.

The leaf area was measured (cm^2) using a leaf-area meter (LI-COR model LI-3100c), considering the leaves from the third leaf pair, from the leaf apex to the base. Fifteen leaves/ plant were evaluated, being three plants/ treatments.

Extraction and chemical analysis of essential oil

To the extraction of essential oil, plants were collected, defoliated, and dried in a forced-air-circulation oven at 40ºC for five days. The essential oils were obtained by the hydrodistillation method in a modified Clevenger apparatus using 3L-flasks, for 140 minutes (Ehlert *et al*., 2006). Three replications were used.

The following variables were evaluated: dry weight (g), essential oil content (50g of dry leaves were distilled), and essential oil yield (mL/plant). The essential oil content was calculated by

$$C(\%) = \frac{v}{w} 100$$

where C is the essential oil content; v is the volume of essential oil extracted from the sample; and w is the dry weight of the leaves.

Chemical analyses were performed at the Gas Chromatography Laboratory of Federal University of Sergipe, using a GC-MS/FID (GCMSQP2010 Ultra, Shimadzu Corporation, Kyoto, Japan) equipped with an autosampler AOC-20i (Shimadzu). Separations were carried out using a capillary column of fused silica Rtx®-5MS Restek (5% - diphenyl- 95% - dimethylpolysiloxane) with 30 m x 0.25 mm of internal diameter (i.d.), 0.25 μm of film thickness, at constant Helium (99.999%) flow rate of 1.0 mL/min. The injector temperature was 280°C, with an injection volume of 1.0 μL (10 mg/mL) and a split ratio of 1:30. The oven temperature was programmed from 50°C (isothermal for 1.5 min), with an increase of 4°C/min to 200°C, and then at 10°C/min to 300°C, ending with an isothermal of 5 min at 300°C. The MS and FID data were simultaneously acquired employing a Detector Splitting System; the split flow ratio was 5:1 (MS:FID). A 0.4 m x 0.15 mm i.d. restrictor tube (capillary column) was used to connect the splitter to the MS detector. A 0.6 m x 0.22 mm i.d. restrictor tube was used to connect the splitter to the FID detector. The MS data (total ion chromatogram, TIC) were acquired in the full scan mode (*m/z* of 40–500) at a scan rate of 0.3 scan/s

using the electron ionization (EI) with electron energy of 70 eV. The injector temperature was 280°C and the ion-source temperature was 200°C. The FID temperature was set to 300°C, and the gas supplies for the FID were hydrogen, synthetic air and helium at flow rates of 30, 300, and 30 mL.min^{-1}, respectively. Quantification of each constituent was estimated by FID peak-area normalization (%). Compound concentrations were calculated from the GC peak areas and they were arranged in order of GC elution.

Compounds were identified according to the comparison of the retention indices found in the literature (Adams, 2017). The retention index was obtained using the Van Den Dool and Kratz (1963) equation in relation to a homologous series of n-alkanes (nC$_7$- nC$_{30}$). Moreover, three spectral libraries of the equipments WILEY8, NIST107, and NIST21

were used, which allowed the comparison of the spectra data with those contained in the libraries, using a similarity index of 80%.

Statistical analysis
Data were submitted to analysis of variance (ANOVA) and, when significant, means were analysed by the Tukey's test at 5% of probability, using the Statistical Software SISVAR® (Ferreira, 2011).

RESULTS
Anatomical and physiological analysis
The results of anatomical analysis are presented in the Table No. 1. The light intensity significantly influenced the leaf anatomical characteristics.

Table No. 1
Palisade and spongy parenchymas thicknesses (µm), leaf blade thickness (µm), central vein thickness (µm), and abaxial and adaxial epidermis thicknesses (µm) of *Varronia curassavica* accessions cultivated in two light conditions

Accessions	Light conditions			
	Full sun	50% shade screen	Full sun	50% shade screen
	-- Palisade parenchyma thickness (µm) --		--- Spongy parenchyma thicknesss (µm) ---	
VCUR-101	66.55bA	37.40bB	39.85aA	40.85cA
VCUR-102	75.55aA	37.70bB	40.15aB	61.90aA
VCUR-201	75.87aA	54.75aB	32.80bA	28.75dA
VCUR-302	62.00cA	34.30bB	31.50bB	52.25bA
VCUR-802	59.40cA	29.90cB	40.75aA	27.80dB
CV a; b (%)	2.00; 3.26		6.96; 5.92	
	------ Leaf blade thickness (µm) ------		------ Central vein thickness (µm) ------	
VCUR-101	101.90bA	74.00cB	559.58bB	594.10dA
VCUR-102	118.10aA	97.70aB	570.00bB	688.20bA
VCUR-201	100.00bA	80.75bB	521.35cB	574.40eA
VCUR-302	98.35bA	84.10bB	728.00aA	653.60cB
VCUR-802	98.55bA	57.50dB	466.20dB	726.15aA
CV a; b (%)	2.82; 2.72		0.49; 0.93	
	--- Abaxial epidermis thickness (µm) ---		---- Adaxial epidermis thickness (µm) ----	
VCUR-101	6.05aA	6.20aA	15.05bA	10.20bB
VCUR-102	5.65aB	7.10aA	21.15aA	10.80bB
VCUR-201	5.95aA	6.50aA	10.05cB	13.60aA
VCUR-302	5.70aA	6.05aA	11.40cA	10.15abA
VCUR-802	6.55aA	3.70bB	9.35cA	7.80bA
CV a; b (%)	13.88; 7.63		3.09; 11.87	

Means followed by the same lowercase letters in the columns and uppercase letters in the rows do not differ by Tukey's test (*p*<0.05). CV: coefficient variation

The variables thickness (PPT) and leaf blade thickness (LBT) presented highest values in full sun condition, for all accessions (Table No. 1; Figure No. 1). The thickest palisade parenchyma was recorded to the accessions VCUR-102 (75.55 μm) and VCUR-201 (75.87 μm) in full sun, and to the accession

VCUR-201 in the protected environment with 50% shade screen (54.75 μm). The thickest leaf blade was recorded to VCUR-102 in both light conditions (118.10 and 97.70 μm, full sun and 50% shade screen, respectively).
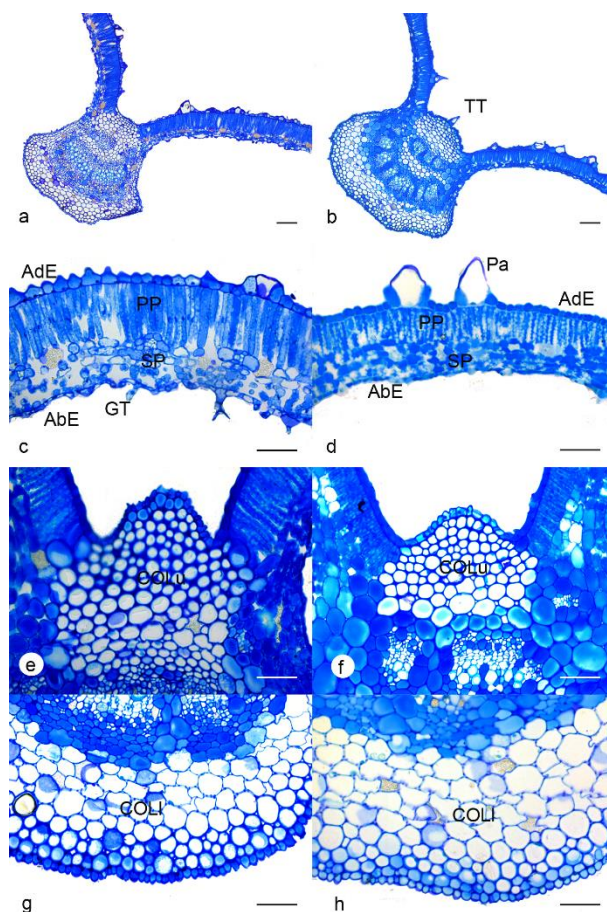

Figure No. 1
Cross sections of leaves of the accession VCUR-201 of *Varronia curassavica* cultivated in two environments, full sun (A, C, E, G) and protected environment with 50% shade screen (B, D, F, H), evidencing the leaf blade thickness (C, D), upper (E, F) and lower (G, H) colequimas. TT = Tectortrichome, GT = Glandular trichome, Pa = Papilla, AdE = adaxial epidermis, AbE = abaxial epidermis, PP = palisade parenchyma, SP = spongy parenchyma, COLu = upper collenchyma, COLl = lower collenchymas, Bars: 50 μm

The response of accessions was different in relation to the two light conditions for the variables: spongy parenchyma thickness (SPT), central vein thickness (CVT), abaxial epidermis thickness (AET) and adaxial epidermis thickness (AET) (Table No. 1). For the variable SPT, the accessions VCUR-101 and VCUR-201 showed no significant difference for the two light intensities. The accessions VCUR-102 and VCUR-302 presented thickest SPT in a protected

environment with 50% shade screen, while the accession VCUR-802 presented thickest SPT in full sun condition. The thickest SPT in full sun condition was observed for the accessions VCUR-101, VCUR-102, and VCUR-802 (39.85, 40.15 and 40.75 μm, respectively) and in protected environment it as observed for VCUR-102 (61.90 μm) (Table No. 1).

The accessions VCUR-101, VCUR-102, VCUR-201 and VCUR-802 presented greater values

of CVT when cultivated in the protected environment with 50% shade screen and the accession VCUR-302 when cultivated in full sun condition. The thickest central vein was observed to VCUR-302 (728.00 μm) in the full sun condition and to VCUR-802 (726.15 μm) in the protected environment with 50% shade screen.

The abaxial epidermis thickness (AbE) in full sun condition had no significant difference between accessions. In the protected environment with 50% shade screen, only the accession VCUR-802 differed from the others, showing the lowest thickness value (3.70 μm). The accessions VCUR-101, VCUR-201 e VCUR-302 presented no significant difference to the two light conditions. The accession VCUR-102 presented greater value of AbE when cultivated under 50% shade screen, and the accession VCUR-802 presented greater value of AbE under full sun condition (Table No. 1). The thickest adaxial epidermis (AdE) was recorded to the accession VCUR-102 in full sun (21.15 μm) and to accessions VCUR-201 and VCUR-302 (13.60 and 10.15 μm, respectively) in protected environment with 50% shade screen. The accessions VCUR-302 and VCUR-802 presented no significant difference to the two light conditions. The accessions VCUR-101 and VCUR-102 presented greater value of AdE when cultivated under full sun condition. The accession VCUR-201 presented greater value of AdE when cultivated under 50% shade screen (Table No. 1).

The levels of a, b and total chlorophyll had no significant difference between the two light conditions, for all accessions. The accessions had no significant difference in the full sun condition to the variables a, b and total chlorophyll, and in the protected environment with 50% shade screen no difference was observed to variable b chlorophyll. The accession VCUR-802 presented the lowest value of a chlorophyll (29.30) in the protected environment with 50% shade screen. Total chlorophyll followed the same trend, with significative difference between the accessions in the protected environment with 50% shade screen (Table No. 2).

Table No. 2

Content of a, b, and total chlorophyll of *Varronia curassavica* accessions cultivated in two light conditions

Accessions	Light conditions					
	Full sun	50% shade screen	Full sun	50% shade screen	Full sun	50% shade screen
	a chlorophyll		b chlorophyll		Total chlorophyll	
VCUR-101	32.95aA	33.45abA	3.80aA	4.45aA	36.75aA	37.85abA
VCUR-102	34.30aA	34.40abA	5.35aA	5.45aA	39.65aA	39.85abA
VCUR-201	32.30aA	35.25aA	4.60aA	6.05aA	36.90aA	41.35aA
VCUR-302	34.15aA	37.40aA	5.40aA	5.55aA	39.55aA	42.95aA
VCUR-802	29.90aA	29.30bA	3.55aA	3.15aA	33.45aA	32.45bA
CV a; b (%)	2.92; 7.53		6.68; 29.75		3.28; 8.78	

Means followed by the same lowercase letters in the columns and uppercase letters in the rows do not differ by the Tukey's test ($p<0.05$). CV: coefficient variation

The two light conditions significantly influenced leaf area (LA) (Table No. 3). When comparing the leaf area between the two light conditions, the protected environment with 50% shade screen provided a larger leaf area in all accessions studied. In full sun, the accession VCUR-201 presented the smallest leaf area (4.05 cm^2). The accession VCUR-802 showed the largest leaf area (18.30 cm^2) in the protected environment with 50% shade screen.

Plant dry weight, essential oil's content, yield, and chemical composition

The light conditions significantly influenced the plant dry weight, the essential oil (EO) content and yield (Table No. 4). In the full sun environment, the highest leaf dry weight was registered for the accessions VCUR-102 and VCUR-201 (35.74 and 40.84 g/plant, respectively). In the protected environment with 50% shade screen, the highest leaf dry weight was recorded for the accessions VCUR-802, VCUR-102 and VCUR-201 (24.26, 19.85 and

19.27 g/plant, respectively). When comparing the two light conditions, the full sun favoured the highest leaf dry weight for the accessions VCUR-102, VCUR-201 and VCUR-302 (Table No. 4).

Table No. 3
Leaf area (cm^2) of *Varronia curassavica* accessions cultivated in two light conditions

Accessions	Light conditions	
	Full sun	50% shade screen
VCUR-101	7.09aB	12.00bA
VCUR-102	6.45aB	8.95cA
VCUR-201	4.05bB	11.50bA
VCUR-302	5.85aB	11.90bA
VCUR-802	5.85aB	18.30aA
CV a; b (%)	6.26; 7.94	

Means followed by the same lowercase letters in the columns and uppercase letters in the rows do not differ by the Tukey's test ($p<0.05$). CV: coefficient variation

For the essential oil content, the highest values were recorded for the accession VCUR-101 (3.96% and 3.32%, in full sun and protected environment with 50% shade screen, respectively). When comparing both levels of light, the protected environment with 50% shade screen favoured only the accession VCUR-302, and no significant difference was observed for the accessions VCUR-102 and VCUR-201 (Table No. 4).

The accessions VCUR-102 and VCUR-201 had higher EO yield per plant in the full sun environment (0.77 and 0.87, respectively), and no significative difference in the EO yield between the accessions in the protected environment with 50% shade screen was observed. The accession VCUR-101 presented higher yield of EO when cultivated under 50% shade screen, while the accessions VCUR-102 and VCUR-201 presented higher yield of EO in the full sun (Table No. 4).

Table No. 4
Plant dry weight, essential oil content and yield of *Varronia curassavica* accessions cultivated in two light conditions

Accessions	Dry weight (g/plant)		Essential oil content (%)		Essential oil yield (mL/plant)	
	Full sun	50% shade screen	Full sun	50% shade screen	Full sun	50% shade
VCUR-101	6.78dB	15.81bA	3.96aA	3.32aB	0.27bcB	0.53aA
VCUR-102	35.74abA	19.85abB	2.13bA	2.35bA	0.77aA	0.47aB
VCUR-201	40.83aA	19.27abB	2.13bA	2.19bA	0.87aA	0.43aB
VCUR-302	33.85bA	18.27bB	1.33cB	2.36bA	0.45bA	0.43aA
VCUR-802	12.59cB	24.26aA	2.04bA	1.41cB	0.26cA	0.34aA
CV a; b (%)	8.75; 9.44		10.21; 7.36		17.00; 16.02	

Means followed by the same lowercase letters in the columns and uppercase letters in the rows do not differ by the Tukey's test ($p<0.05$). CV: coefficient variation

The essential oils, for all accessions, consisted of sesquiterpenes. Based on the chemical analysis, 18 major compounds were selected and listed in order of elution (Table No. 5).

Table No. 5
Chemical composition of the essential oil of *Varronia curassavica* accessions cultivated in two light conditions

	Compounds (RRI-l/RRI-o)					
Accessions	(*E*)-caryophyllene (1417/ 1423)		α-humulene (1452/ 1457)		ar-curcumene (1479/ 1481)	
	Full sun	50% shade screen	Full sun	50% shade screen	Full sun	50% shade screen
VCUR-101	3.52bcB	5.80bA	1,26bB	1.85bA	0.00cA	0.00cA
VCUR-102	1.47cA	2.08cA	0,61cA	0.84cA	0.00cA	0.00cA
VCUR-201	5.60bA	4.33bA	1,83bA	1.42bA	0.00cA	0.00cA
VCUR-302	12.22aB	17.90aA	3,71aB	5.11aA	4.79aA	2.57aB
VCUR-802	4.64bB	6.36bA	1,52bB	2.01bA	0.62bA	0.69bA
CV-a (%)	15.21		12.91		16,83	
CV-b (%)	13.25		12.23		15,66	
	germacrene D (1484/ 1484)		α-zingiberene (1493/ 1496)		bicyclogermacrene (1500/ 1502)	
	Full sun	50% shade screen	Full sun	50% shade screen	Full sun	50% shade screen
VCUR-101	1.92aB	2.63bA	0.00cA	0.00cA	6.97aB	10.87aA
VCUR-102	0.52cA	0.80dA	0.00cA	0.00cA	2.44bA	4.14bA
VCUR-201	0.69bcA	0.62dA	0.00cA	0.00cA	3.60bA	5.30bA
VCUR-302	2.49aB	4.86aA	32.38aB	38.04aA	3.33bA	3.52bA
VCUR-802	1.13bB	1.75cA	3.32bA	3.32bA	5.05abA	5.54bA
CV-a (%)	18.39		12.37		15.59	
CV-b (%)	13.39		11.86		24.63	
	δ-cadinene (1522/ 1524)		β-sesquiphellandrene (1521/ 1527)		shyobunone IV (1560/ 1564)	
	Full sun	50% shade screen	Full sun	50% shade screen	Full sun	50% shade screen
VCUR-101	3.92aB	4.80aA	0.00cA	0.00cA	6.92bA	2.96bB
VCUR-102	2.27bB	4.68aA	0.00cA	0.00cA	13.93aA	6.50aB
VCUR-201	0.00cA	0.00cA	0.00cA	0.00cA	0.00cA	0.00cA
VCUR-302	0.00cA	0.00cA	14.06aA	14.45aA	0.00cA	0.00cA
VCUR-802	0.00cA	0.00cA	1.60bA	1.77bA	0.00cA	0.00cA
CV-a (%)	5.68		5.61		10.21	
CV-b (%)	14.70		9.26		11.42	
	germacrene D-4-ol (1574/ 1575)		spathulenol (1577/ 1587)		7-methyl-3-methylene-10-(1-propyl)-7-cyclodecen-1-one (1613/ 1621)	
	Full sun	50% shade screen	Full sun	50% shade screen	Full sun	50% shade screen
VCUR-101	0.50bA	0.41bA	3.50bA	1.82bB	31.02aB	33.98aA
VCUR-102	0.00bA	0.00bA	5.16aA	3.17aB	32.40aA	33.56aA
VCUR-201	5.37aB	8.30aA	1.08dA	0.63cB	0.00bA	0.00bA
VCUR-302	0.00bA	0.00bA	2.17cA	0.55cB	0.00bA	0.00bA
VCUR-802	0.00bA	0.00bA	1.73cA	1.72bA	0.00bA	0.00bA
CV-a (%)	29.47		13.49		6.20	
CV-b (%)	24.86		10.97		11.15	
	epi-α-muurolol (1640/ 1648)		α-cadinol (1652/ 1663)		ar-turmerone (1668/ 1672)	

	Full sun	50% shade screen	Full sun	50% shade screen	Full sun	50% shade screen
VCUR-101	0.00bA	0.00bA	0.99bA	1.59bA	0.00bA	0.00bA
VCUR-102	0.00bA	0.00bA	0.89bB	2.48bA	0.00bA	0.00bA
VCUR-201	2.92aB	5.89aA	3.88aB	9.10aA	0.00bA	0.00bA
VCUR-302	0.00bA	0.00bA	0.00bA	0.00cA	0.00bA	0.00bA
VCUR-802	0.00bA	0.00bA	0.00bA	0.00cA	24.21aA	24.34aA
CV-a (%)	16.33		10.58		6.80	
CV-b (%)	24.11		22.50		9.27	
	curlone (β-turmerone) (1701/ 1706)		shyobunol (1688/ 1713)		(2E,6E)-methylfarnesoate (1783/ 1785)	
	Full sun	50% shade screen	Full sun	50% shade screen	Full sun	50% shade screen
VCUR-101	0.00bA	0.00bA	0.00bA	0.00bA	0.00bA	0.00bA
VCUR-102	0.00bA	0.00bA	0.00bA	0.00bA	0.00bA	0.00bA
VCUR-201	0.00bA	0.00bA	28.44aA	23.04aB	5.81aA	2.44aB
VCUR-302	0.00bA	0.00bA	0.00bA	0.00bA	0.00bA	0.00bA
VCUR-802	4.92aB	5.50aA	0.00bA	0.00bA	0.00bA	0.00bA
CV-a (%)	2.73		2.88		24.29	
CV-b (%)	15.47		19.36		18.04	

Means followed by the same lowercase letters in the columns within each environment, uppercase letters in the rows between time do not differ by the Tukey's test ($p<0.05$). RRI-l: relative retention index – literature; RRI-o: relative retention index - observed. CV: coefficient variation

The accession VCUR-101 presented the highest content of (*E*)-caryophyllene, α-humulene, germacrene D, bicyclogermacrene, δ-cadinene and 7-methyl-3-methylene-10-(1-propyl)-7-cyclodecen-1-one when cultivated in the protected environment with 50% shade screen. The highest content of shyobunone IV and spathulenol was observed when the accession VCRU-101 was cultivated in full sun (Table No. 5).

The environment with 50% shade screen favored the increase of the compounds δ-cadinene and α-cadinol, and the reduction of the compounds shyobunone IV and spathulenol for the accession VCUR-102. The accession VCUR-201 presented the highest content of the compounds germacrene D-4-ol, epi-α-muurolol and α-cadinol in the protected environment with 50% shade screen. In this light condition, it was observed reduction of the compounds spathulenol, shyobunol and (2*E*,6*E*)-methylfarnesoate, for the accession VCUR-201 (Table No. 5).

The increase of the compounds (*E*)-caryophyllene, α-humulene, germacrene D and α-zingiberene was observed when the accession VCUR-302 was cultivated in the environment with 50% shade screen. On the other hand, in this light condition, it was observed reduction of the compounds ar-curcumene and spathulenol, for the accession VCUR-302 (Table No. 5).

The accession VCUR-802 presented the highest content of the compounds (*E*)-caryophyllene, α-humulene, germacrene D and curlone (β-turmerone) when cultivated with 50% shade screen (Table No. 5).

The presence of exclusive compounds was observed for the accessions VCUR 201 [epi-α-muurolol, shyobunol and (2*E*,6*E*)-methylfarnesoate] and VCUR-802 [ar-turmerone and curlone (β-turmerone)] (Table No. 5).

DISCUSSION
Anatomical and physiological analysis
The leaf plasticity was affected by the light conditions (Figure No. 1) and genotypes (accessions) (Table No. 1). Leaf anatomy can influence directly light capture by its leaf thickness, and by the differentiation of palisade and spongy parenchyma (Zheng & Labeke, 2017). The accessions cultivated in full sun had thicker palisade parenchyma and more elongated and juxtaposed cells with fewer intercellular spaces (Figure No. 1c). Conversely, the accessions cultivated in the protected environment with 50% shade screen had lower values for PPT, presenting compacted cells (Figure No. 1d). The

palisade parenchyma gets thicker due either to cells elongation or addition of a new layer (Lammers *et al*., 2008; Boeger *et al*., 2009), as was observed in *V. curassavica*. Cell elongation occurs with the increase in soluble sugars and the decrease in the starch, which in turn increases the cellular osmotic potential and increases the vacuoles size (Taiz & Zieger, 2013). The addition of a new layer occurs when the cells division begins early at a higher rate, ceasing more briefly and resulting in two or more palisade cell layers.

For the spongy parenchyma, the accessions VCUR-102 and VCUR-302 cultivated in the protected environment with 50% shade screen presented larger intercellular spaces (Figure No. 1c). The intercellular spaces in spongy parenchyma increases the reflection and refraction of light between cells, the proportion of light reflected for the palisade parenchyma, and the amount of light intercepted per chlorophyll unit in the cells (Valladares *et al*., 2012).

Similarly, variations in the parenchymas thicknesses were detected in *Colocasia esculenta*, with a reduction in the palisade and spongy parenchymas when cultivated in the protected environment with 50% shade screen (34.14% and 23.30%) (Gondim *et al*., 2008). *Thymus vulgaris* plants presented reduced palisade and spongy parenchymas when cultivated at 30% luminosity intensity (Salgado *et al*., 2012). *Chrysanthemum* presented thicker leaves when cultivated under $100 \ \mu mol \ m^{-2} \ s^{-1}$ due increment in the size of palisade cells, and due to a major number of spongy parenchyma layers (Zheng & Labeke, 2018).

The increase of the palisade parenchyma in the full sun condition resulted in a thicker leaf blade (Figure No. 1c), showing a mean increase of 34.14% in relation to the accessions cultivated in the protected environment with 50% shade screen (Table No. 1). This result reflects an increase in the volume of mesophyll per leaf area, which may favour the structural photosynthetic mechanism. This mechanism increases the diffusion of CO_2 in the intercellular space, facilitates the CO_2 diffusion by the rubisco enzyme (ribulose 1,5-bisphosphate carboxylase/oxygenase), and regulates light intensity, consequently protecting the photosynthetic apparatus and improving its efficiency (Terashima *et al*., 2006; Valladares *et al*., 2012; Ribeiro *et al*., 2018).

Similar results were obtained in leaves of *Chenopodium album*, whose mesophyll increased significantly when subject to high radiation (Oguchi *et al*., 2003). *Acacia koa* had an increase in the leaf blade in function of irradiance intensity, except for the spongy parenchyma (Craven *et al*., 2010).

The accessions cultivated in the protected environment with 50% shade screen, except the accession VCUR-302, presented higher values for CVT in relation to the full sun environment (Figure No. 1A and Figure No. 1B, respectively). The thicker central vein may be directly related to the increase of the upper (Figure No. 1E and Figure No. 1F) and lower (Figure No. 1G and Figure No. 1H) collenchyma cells. Collenchyma cells increased to up to 29% in plants of *Colocasia esculenta* cultivated in the protected environment with 50% shade screen (Gondim *et al*., 2008). Usually, plants cultivated in a shaded environment present larger, less thick, and less heavy leaves per unit area when compared with those cultivated in full sun (Craven *et al*., 2010).

Plants kept under greater irradiance have the epidermis of one or both surfaces thicker than the plants cultivated in the shade environment (Lee *et al*., 2000), this fact was observed to the accessions of *V. curassavica*. The adaxial epidermis of accessions VCUR-101 and VCUR-102 cultivated in full sun was thicker than those cultivated in the protected environment with 50% shade screen (Figure No. 1C and Figure No. 1D).

The plasticity of the abaxial and adaxial epidermis varies according to the luminosity, cultivation time (Morais *et al*., 2004; Gratani *et al*., 2006), and genotype, as was observed in this study. So, the rise of radiation tends to increase leaf thickness in *V. curassavica,* as noted by Ribeiro *et al*. (2018) in *Pogostemon cablin*. This tissue protects the plant surface and controls gas exchanges, including water vapor. Plants with a thicker epidermis provide better protection to the mesophyll against several injuries (Castro *et al*., 2009).

The abaxial and adaxial epidermis of the leaves of *Aloysia gratissima* also varied when cultivated under low lighting conditions (Pinto *et al*., 2007). The leaves of *Mikania glomerata* presented no change in the epidermis thickness when cultivated under different lighting conditions (Espindola Junior *et al*., 2009), as was observed for the accession VCUR-302.

The values of a, b, and total chlorophyll did not change, suggesting that both light conditions were sufficient to maintain the integrity and the optimal functioning of the molecules, and that the lower light intensity did not limit the efficiency of photosystem II (Zheng & Labeke, 2018). This is because

chlorophylls can undergo photo-inhibition and photo-oxidation processes under low light conditions and degradation process under high light conditions (Taiz & Zieger, 2013). Additionally, the leaf area does not affect the efficiency of light capture by the pigments, and the leaf absorptance is simply related to the chlorophyll content per unit leaf area (Evans & Poorter, 2001), as noted in this study.

Accessions cultivated in the protected environment with 50% shade screen had higher LA when compared with those cultivated in the full sun environment (Table No. 3). These results are in line with those observed by Alexandre *et al.* (2018) and Feijó *et al.* (2014) in *Talinum triangulare* and *Varronia. curassavica*, respectively. Smaller leaf area may be a strategy of the plant to reduce water loss by transpiration since the smaller the contact area with light irradiance, the lower is the contact with the wind and heat, leading to lower water loss. Moreover, smaller leaf area increases the number of chloroplasts and the number of photosynthetic enzymes, and thereby enhances the photosynthetic capacity per unit leaf area (Evans & Poorter, 2001). Conversely, the increase in the leaf area in plants cultivated under low irradiance is a way to increase the light energy capture and to achieve greater photosynthetic efficiency (Taiz & Zieger, 2013; Feijó *et al.*, 2014) to compensate the reduction of the palisade and spongy parenchymas.

Plant dry weight, essential oil's content, yield, and chemical composition

The greater dry weight observed for the accessions VCUR- 102, VCUR-201 and VCUR-302 cultivated in full sun may be related to the increase of leaf blade tissues (Table No. 4). Thicker leaf blade tissues allow a greater and faster CO_2 diffusion by the rubisco enzyme, favouring the action of the carboxylase enzyme, consequently promoting greater carbon fixation and gain (Terashima *et al.*, 2006). For these accessions, the biomass response is a result of additional light energy provided for photosynthesis activity (Zheng & Labeke, 2018). Moreover, this was not observed for the accessions VCUR-101 and VCUR-802, showing that the biomass response is dependent of the genotype.

Significant differences were observed in essential oil content and yield to different light conditions and accessions of *V. curassavica* (Table No. 4). The genetic variability influences the essential oil yield (Taiz & Zeiger, 2013), as noted in this study. The essential oil content of accessions

VCUR-102 and VCUR-201 cultivated in the protected environment with 50% shade screen did not differ to that of plants cultivated in full sun, even with less thick leaf blade and lower leaf dry weight. This result was registered for *V. curassavica* (Feijó *et al.*, 2014), *Ocimum basilicam* (Chang *et al.*, 2008), and *Lippia citriodora* (Gomes et al., 2009). Plants cultivated in full sun were expected to have higher essential oil content since the essential oil yield may be related to the frequency of globular trichomes in the leaves of *V. curassavica*, which, in turn, is directly associated with the increase in irradiation (Feijó *et al.*, 2014).

The essential oil yield is the direct result of the essential oil content and the plant biomass yield (Costa *et al.*, 2010). In *V. curassavica*, the full sun environment provided higher essential oil yield than the protected environment with 50% shade screen, for the accessions VCUR-102 and VCUR-201. The variation in essential oil content and yield in function of different light conditions, prove the importance of the light over different biosynthetic routes of secondary metabolism in plants (Araújo *et al.*, 2018).

The synthesis of a chemical compound is linked to the presence of different enzymes, produced during the plant development, that catalyse the reactions (Sangwan *et al.*, 2001; Montanari *et al.*, 2011). These new compounds might explain the variations in the content of each chemical compound and these variations are obtained from the different environmental conditions to which the plants were initially subject. Thus, the synthesis of the essential oils is influenced by internal (genetic) and external (environmental) factors that direct the type of compounds to be produced and their respective contents (Morais, 2009). It can be inferred that, in this study, the genetic constitution of the accessions and the accessions x light conditions interaction influenced the content of the chemical compounds of the essential oil (Table No. 5). The differences observed reveal the strong influence of the genotype on light regulation of the synthesis of essential oils, which vary in function of the adaptation or tolerance of the accessions to different light patterns (Araújo *et al.*, 2018).

The higher biosynthesis of a given compound may reduce another compound due to the biosynthetic correlation between them. For instance, spathulenol may be a product of the bicyclogermacrene oxidation (Njoroge *et al.*, 2003). The bicyclogermacrene content did not differ between the two environments, except for the

accession VCUR-101, which had a 35.87% increase in the content of this compound when cultivated in a shade screen environment. However, spathulenol had the highest content when the plant is cultivated in full sun environment. This fact suggests that the environments did not interfere with the bicyclogermacrene oxidation.

α-humulene is the chemical marker to the pharmaceutical industry for quality control of *V. curassavica* essential oil (Marques et al., 2019). The essential oil of all accessions in both light conditions presented this compound (Table No. 5), but higher content was observed in the environment with 50% shade screen. *V. curassiva* essential oil must contain a minimum content of 2.3% to 2.9% v/v of α-humulene. (Quispe-Condori *et al*., 2008). This amount was determined only in the accession VCUR-302.

The content of (*E*)-caryophyllene compound followed the same tendency of α-humulene compound. The accessions cultivated in the environment with 50% shade screen presented higher content when compared to the full sun condition. The accessions VCUR-102 and VCUR-201 showed no significant difference for the two light conditions, for both compounds. This increase of (*E*)-caryophyllene

on the 50% shade condition is probably related to plant stress, resulting from the (*E*)-caryophylene oxidation (Feijó *et al*., 2014).

CONCLUSION
The light condition influence significantly the anatomy, physiology and essential oil composition and yield of *Varronia curassavica* accessions. Additionally, the genetic constitution of the accessions and the accessions x light conditions interaction influenced the content of the chemical compounds of the essential oil; and the cultivation of the accessions in the environment with 50% shade screen favored the increase of the chemical markers of *Varronia curassavica* accession.

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