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Baccharis dracunculifolia DC: Climate-Driven Metabolomic Variability in Essential Oils, Trichomes, and Antifungal Activity

Paulo H. Furlan, Júlio C. R. L. Silva, Roselaine Facanali, Daniela M. Soares, Mariana N. F. Cabral, Tatiane M. Rodrigues, Aline R. Martins, Eduardo M. da Gloria, and Marcia O. M. Marques*

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ABSTRACT: *Baccharis dracunculifolia* DC. is a medicinal and aromatic plant species widely distributed in South America, predominantly in Brazil. It has been traditionally used in folk medicine and is the primary botanical source of Brazilian green propolis. The plant produces essential oils (EOs) in the glandular trichomes of its leaves, which exhibit pharmacological properties, including anti-inflammatory, antioxidant, and antimicrobial activities. Furthermore, these EOs also show potential for controlling agricultural pests and diseases. As a nondomesticated species, understanding its natural population dynamics and environmental adaptations is critical for selecting high-value genotypes and enhancing its commercial and ecological potential. This study investigated seasonal variation in glandular trichome density and EO chemical composition across dry and rainy seasons as well as their antifungal properties. While trichome density showed no significant seasonal variation, the EO yield increased during the rainy season (0.70 ± 0.16%). Comprehensive two-dimensional gas chromatography (GC×GC) analysis resulted in 88 compounds, with (*E*)-nerolidol, *β*-pinene, limonene, spathulenol, and bicyclogermacrene as the predominant constituents. Coelutions observed in one-dimensional GC were resolved using GC×GC, enabling the identification of minor season-specific compounds that chemically distinguished dry and rainy seasons. Antifungal assays revealed intrapopulation and seasonal variability in the inhibition of *Fusarium graminearum*, *Fusarium verticillioides*, and *Aspergillus nomius*. The compounds *p*-cymene, *γ*-muurolene, and *α*-cadinol exhibited the strongest correlation with the antifungal activity. The most successful EOs for antifungal activity were from genotypes 1BD02, against *F. graminearum* and *A. nomius*, and 1BD06, against *F. verticillioides*, both obtained in the dry season. These findings provide a framework for integrating ecophysiology and metabolomics to guide genotype selection of *B. dracunculifolia*.

KEYWORDS: *comprehensive two-dimensional gas chromatography (GC×GC), natural antifungals, storage fungi, ecophysiology, secretory structures, minor constituents*

1. INTRODUCTION

Baccharis dracunculifolia DC., a species in the family Asteraceae, is a medicinal and aromatic plant native to Brazil that is commonly used in folk medicine. It is known popularly as "alecrim-do-campo" and "vassourinha" and is predominantly distributed in the Pampa, Cerrado, and Atlantic Forest biomes.[1] The species plays a significant role in beekeeping, serving as the primary botanical source of green propolis from *Apis mellifera*. This natural product possesses high added value due to its antiviral, antitumor, and antibiotic properties.[2] Multicellular glandular trichomes, found in the leaves, are responsible for the secretion of resins and essential oils (EOs).[3,4] Essential oils, in particular, have been demonstrated to possess significant potential in various applications, including cosmetics, pharmaceutical products, and agricultural applications, such as pest and disease control.[5−10]

The chemical composition of *B. dracunculifolia* EOs is characterized by the presence of sesquiterpenes, such as (*E*)-nerolidol, germacrene D, bicyclogermacrene, (*E*)-*β*-caryophyllene, and spathulenol as well as high concentrations of monoterpenes, such as *β*-pinene and limonene.[6,11] The EOs are highly valued by the flavor and fragrance industry for their content of potent aromatic compounds—such as cabreuva oxides and *β*-damascenones—which are present in low concentrations in this species, yet contribute significantly to its aroma profile.[11,12]

Fungal species such as *Fusarium graminearum*, *Fusarium verticillioides*, and *Aspergillus nomius* are among the main fungal species associated with grain contamination during storage, causing both quantitative and qualitative losses in agricultural production. These fungi are also major producers of highly toxic mycotoxins, which make grains unsuitable for human and animal consumption even at low concentrations.[10,13] With the increasing resistance of these microorganisms to antibiotics, food preservatives, and pesticides, there is an urgent need for new products with antifungal and antibacterial activity. The antagonistic properties that EOs have against these microorganisms make them a promising alternative, in addition to being an environmentally friendly solution.[14,15] In this context, studies involving *B. dracunculifolia* have demonstrated signifi-

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cant potential for the agricultural sector in the control of pests and diseases.[10,13,16]

Despite being widely used in several industries for their biological potential, the chemical profile of EOs is subject to variation due to genetic factors, as well as intra- and interpopulation differences.[11,17−19] Additionally, abiotic factors, including altitude, circadian rhythm, soil physical properties, light incidence, water availability, temperature, and seasonality, have been demonstrated to influence the secondary metabolite composition of EOs.[18−21] The synthesis of EOs in plant tissues occurs in secretory structures such as glandular trichomes, which can be present in the vegetative and reproductive organs of aromatic plants.[22] Climatic variation has been shown to influence the density and functionality of these glandular trichomes.[23,24] While previous studies have addressed the chemical composition, yield, and antimicrobial activities of *B. dracunculifolia* EOs,[5−11] research on the influence of seasonal variation on the density of secretory structures in this species remains unexplored. The chemical complexity of *B. dracunculifolia* EOs, characterized by coeluting compounds in conventional analyses, demands advanced metabolomic tools. In this context, we employed comprehensive two-dimensional gas chromatography (GC×GC−MS/FID)—the first application of this technique to *B. dracunculifolia*—which overcomes resolution limitations of traditional GC−MS (as detailed below). This approach revealed season-driven shifts in the metabolic profile linked to secretory structure dynamics.

The chemical characterization of EOs has benefited from significant advancements in recent years in separation and analysis techniques for volatile substances. This is particularly evident in the analysis of complex matrices that exhibit coelutions, such as the EOs of *B. dracunculifolia*. As applied in this study, comprehensive two-dimensional gas chromatography (GG×GC) has emerged as the most effective separation technique.[25,26] In contrast to one-dimensional gas chromatography (1D-GC), which uses a single column for separation, the GC×GC system is characterized by two columns of different polarity, i.e., orthogonal, connected by a modulator. Substances of interest are detected in the first column, analogous to the configuration adopted in GC, and subsequently directed to a second column of shorter length and higher polarity.[27,28] This separation process has led to gains in resolution and sensitivity, with an increase in the number of substances detected, which in most cases is twice as high as that observed using the one-dimensional system.[25,26,28] These advantages position GC×GC as a powerful metabolomic tool for EO studies, allowing not only the identification but also the association of the largest possible number of substances with potential applications of interest such as antifungal properties.

The hypothesis of our study was that dry and rainy seasons influence the density of glandular trichomes of *B. dracunculifolia* as well as the volatile profiles and antifungal activities of its EOs. In this context, the objective of this work was to investigate seasonal variation in the density of leaf glandular trichomes and in the chemical composition of EOs from genotypes of a *B. dracunculifolia* population using GC×GC−MS/FID analysis as well as to assess their antifungal activity against storage fungi during different collection periods (dry and rainy seasons).

2. MATERIALS AND METHODS

2.1. Plant Material. Plant material of *B. dracunculifolia* was collected in an area of Cerrado located in the municipality of Águas de Santa Bárbara, state of São Paulo, Brazil (geographic coordinates: 22° 59′ 46″ S and 49° 18′ 32″ W), with the authorization of the Brazilian Ministry of the Environment (registration number A4C3B2F). The climate of this region is Cwa, according to the Köppen-Geiger classification.[29] Vegetative aerial stems were sampled from 15 marked individuals ($n = 15$) of a natural population, with the same plants being sampled in both the dry and rainy seasons (6 September 2021 and 21

Table 1. Nomenclature Given to the Genotypes of the *B. dracunculifolia* Population Sampled in the Dry and Rainy Seasons in the Municipality of Aguas de Santa Barbara, State of São Paulo, Brazil[a]

individuals	dry season	rainy season
B. dracunculifolia—01 (M;C)	1BD 01	2BD 01
B. dracunculifolia—02 (M;C)	1BD 02	2BD 02
B. dracunculifolia—03 (M;C)	1BD 03	2BD 03
B. dracunculifolia—04 (M;C)	1BD 04	2BD 04
B. dracunculifolia—05 (M;C)	1BD 05	2BD 05
B. dracunculifolia—06 (M;C)	1BD 06	2BD 06
B. dracunculifolia—07 (M;C)	1BD 07	2BD 07
B. dracunculifolia—08 (M;C)	1BD 08	2BD 08
B. dracunculifolia—09 (M;C)	1BD 09	2BD 09
B. dracunculifolia—10 (M;C)	1BD 10	2BD 10
B. dracunculifolia—11 (C)	1BD 11	2BD 11
B. dracunculifolia—12 (C)	1BD 12	2BD 12
B. dracunculifolia—13 (C)	1BD 13	2BD 13
B. dracunculifolia—14 (C)	1BD 14	2BD 14
B. dracunculifolia—15 (C)	1BD 15	2BD 15

[a]M = Individuals selected for scanning electron microscopy (SEM); C = Individuals selected for EO chemical composition analysis.

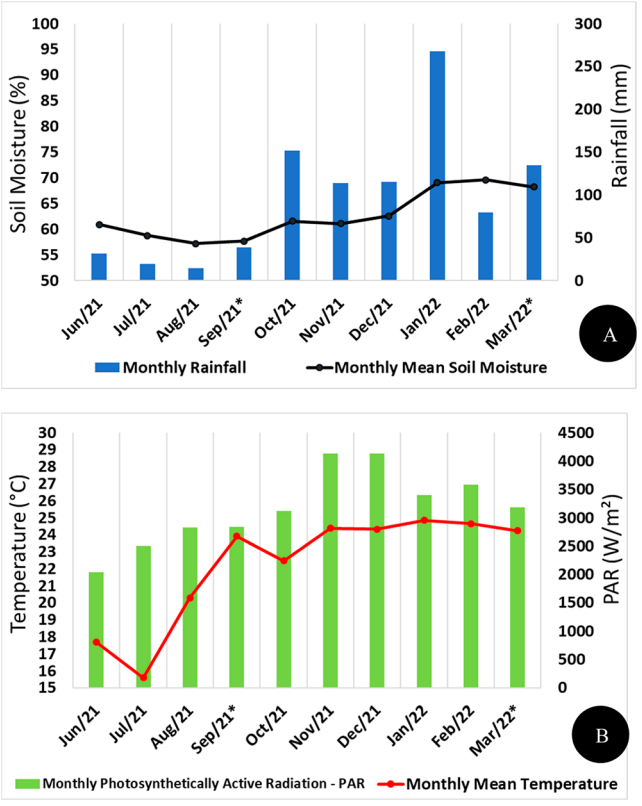

Figure 1. (A) Monthly rainfall (mm) and monthly mean soil moisture at a depth of 1 m (%). (B) Monthly mean temperature (°C) and monthly photosynthetically active radiation, PAR (W/m²), from June 2021 to March 2022. Data were collected from the NASA Power database in December 2022. * = collection months.

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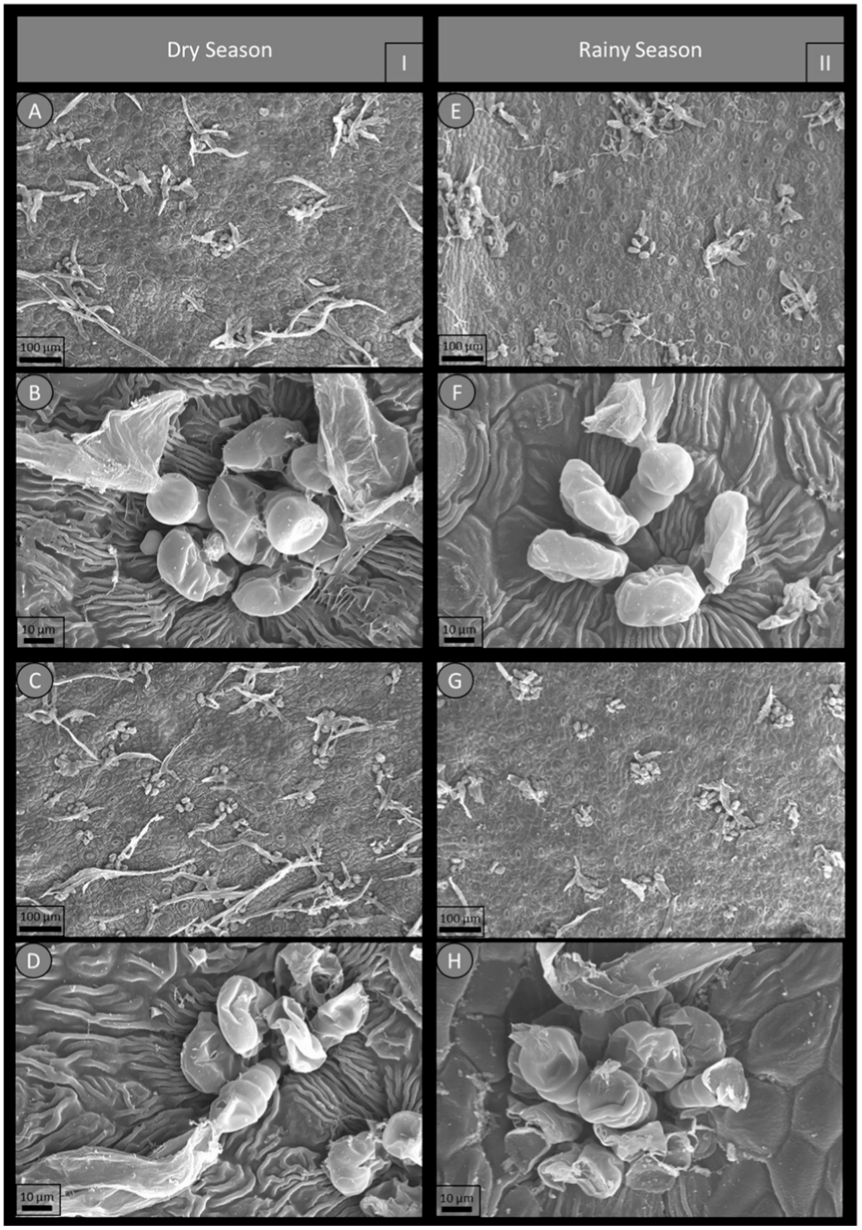

Figure 2. Scanning electron microscopy (SEM) of *B. dracunculifolia* leaves collected in the dry (I) and rainy (II) seasons; 1BD08 and 2BD08, respectively, showing glandular and nonglandular trichomes. (A,B,E,F) abaxial leaf blade surface. (C,D,G,H) adaxial leaf blade surface.

March 2022, respectively). All samples were collected during the morning (7:00−10:00 a.m.), resulting in a total of 30 samples. Individuals 1 to 15 received a specific nomenclature for each collection (Table 1).

2.2. Climatological Data. Climatological data—including temperature (°C), soil moisture (%) in the root zone, rainfall (mm), and photosynthetically active radiation (W/m^2)—were obtained from the NASA Power database between June 1, 2021, and March 31, 2022 (Figure 1).[30]

2.3. Essential Oil Extraction. Leaves of *B. dracunculifolia* were manually removed from the collected stem parts, and the EOs were extracted by hydrodistillation in a Clevenger apparatus using approximately 80 g of fresh leaves for 2 h. The EOs were stored in 5 mL glass vials, which were preserved at 4 °C and protected from light. Some fresh leaves were oven-dried at 60 °C, and the EO yield was expressed on a dry basis in triplicate.

2.4. Essential Oil Chemical Composition by Comprehensive Two-Dimensional Gas Chromatography−Mass Spectrometry (GC×GC−MS/FID). Essential oil samples were diluted in ethyl acetate (chromatographic grade, 1 mg·mL^{-1}; Merck, Darmstadt, Germany). An aliquot of 1 μL of solution was injected at a split ratio of 20:1. The injector was kept at 220 °C. Helium (purity 5.0, White Martins SA, Brazil) was used as a carrier (0.5 mL·min^{-1}) and auxiliary gas (20 mL·min^{-1}). The analysis was performed on a TRACE 1310 gas chromatograph equipped with a flame ionization detector (FID) and an ISQ mass spectrometer (Thermo Fisher Scientific). The mass spectrometer (MS) operated in full scan mode with an acquisition range of 40 to 450 m/z. Electron ionization (EI) was used at 70 eV. The transfer line temperature was maintained at 230 °C. The FID was operated at 250 °C.

The first and second columns consisted of a Rtx-5 column (20 m × 0.18 mm × 0.2 μm) (^{1}D) and a Rtx Wax column (5 m × 0.25 mm × 0.25 μm) (^{2}D), respectively, both from Restek Corporation (Bellefonte, PA, USA). The temperature program was set from 60 to 240 °C at 3 °C·min^{-1}. The effluent of the second column was passively split using a SilFlow 3 Port GC splitter and two deactivated capillaries (5 m × 0.18 mm and 5 m × 0.32 mm), resulting in a split ratio of 1:3 for MS and FID, respectively.[31,32]

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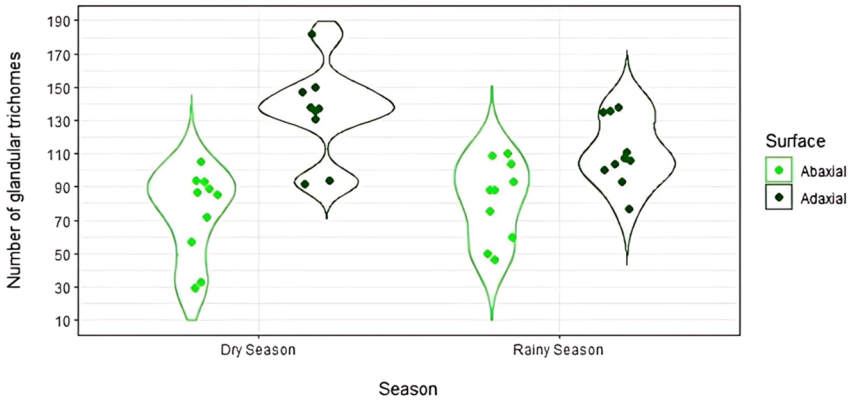

Figure 3. Violin plot of the density of glandular trichomes observed on the abaxial and adaxial surfaces of leaves of *B. dracunculifolia*. Two-way ANOVA: glandular trichome density between leaf surfaces ($F = 27.9775$, $p = 0.0000067$); glandular trichome density between seasons ($F = 1.6623$, $p = 0.2053$).

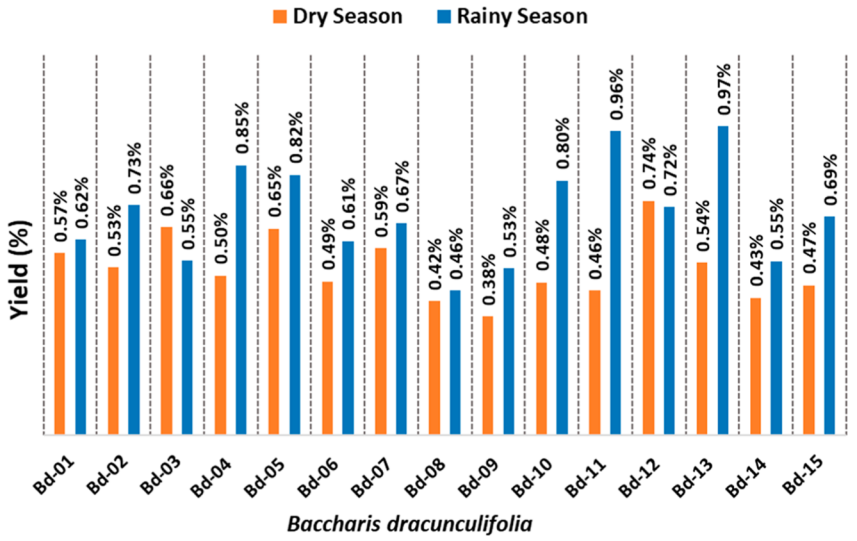

Figure 4. Essential oil yield of 15 individuals of *B. dracunculifolia* collected in September 2021 and March 2022 (dry and rainy seasons, respectively); *t*-test (paired): $t = -4.0038$; $p = 0.0006531$.

The modulation period was set to 6.0 s with a reinjection (flush) pulse of 250 ms. Chromeleon software (Thermo Scientific, Waltham, MA, USA) was used for data acquisition. Data processing was performed by using GC Image software (Zoex, Houston, TX, USA).

The chemical constituents were identified by comparative analysis of the mass spectra of the substances with those of the National Institute of Standards and Technology libraries (NIST 14) and Flavor & Fragrance Natural & Synthetic Compounds (FFNSC3), and of linear retention indices (LRIs) with those of compounds in the literature.[33] The experimental LRI for each compound was obtained by injecting a mixture of *n*-alkanes (C_9−C_{20}, Sigma USA) under the same chromatographic conditions as the samples, applying the Van den Dool and Kratz equation.[34]

2.5. Glandular Trichome Density. Glandular trichome density was evaluated by scanning electron microscopy (SEM) using fully expanded leaves obtained from the third stem node of 10 individuals in each collection (season). Portions of the median region of the leaf were fixed in Karnovsky's solution,[35] dehydrated in an ethanol series, dried at a critical point (Leica EM CPD300), and attached to an aluminum support with double-sided carbon tape. After metallization with a 30−40 nm gold layer in a sputtering metallizer (Quorum Q150T E), the samples were analyzed under a scanning electron microscope (Zeiss, EVO LS 15), operated at 20 kV. Trichome density was calculated on the adaxial and abaxial sides of the leaf blade from electron micrographs taken at 250× magnification (1200 μm × 800 μm).

2.6. Evaluation of the Antifungal Activity of *B. dracunculifolia* Essential Oils against *A. nomius*, *F. graminearum*, and *F. verticillioides*. Antifungal activity was evaluated by measuring growth restriction by direct contact of fungi with the medium containing the EOs. Preliminary screening of the EO samples was performed to identify those with the greatest antifungal activity against the evaluated species. The treatment medium was prepared by manually homogenizing a mixture containing 30 mL of autoclaved Potato Dextrose Agar (PDA, KASVI), 600 μL of the emulsifier Etherius (developed at ESALQ−USP), and 60 μL of *B. dracunculifolia* EO, corresponding to a final concentration of 2 mL·L^{-1}. Approximately 10 mL of this mixture was poured into each Petri dish (15 × 60 mm). The treatments were performed in triplicates. A mixture containing only PDA and emulsifier (30 mL and 600 μL, respectively) was prepared as a negative control, with 10 mL distributed on each dish.

Evaluations involved placing a sterile filter paper disk (5 mm in diameter) in the center of the solidified culture medium, to which 5 μL of spore solution was added. A single isolate of *A. nomius*, *F. graminearum*, and *F. verticillioides* was used for these evaluations. The *Fusarium* species were isolated at the State University of Maringá (UEM), while the *Aspergillus* strain was isolated from Brazil nuts at the Institute of Biomedical Sciences of the University of São Paulo (USP). All fungal isolates were provided to the Food Microbiology Laboratory of the Department of Agroindustry, Food and Nutrition at the Luiz de Queiroz College of Agriculture, University of São Paulo (ESALQ−USP), where the antifungal assays were conducted. Spore solutions

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were prepared from colonies of each species grown on PDA for 14 days. For this purpose, about 15 mL of sterile water was added to the plates on which the fungus was growing and the surface was scraped with a Drigalski loop to release the spores. The spore count in the inoculum solution was standardized using a hemocytometer (Neubauer chamber) to obtain densities between 3 and 30 × 10^{-6} spores/mL. After inoculation, the dishes were incubated at 25 °C without a photoperiod. Observed radial fungal growth was measured, using a caliper, from the center of the plate at 72 and 144 h after inoculation of the fungus. After these measurements, percent growth inhibition caused by the EOs was calculated from the ratio of the average diameter of the observed fungal growth on the plates to the average diameter of the observed fungal growth on the negative control, multiplied by 100.

2.7. Statistical Analysis. Data on the yield and antifungal activity of the EOs were subjected to a paired *t*-test at a 5% significance level. Mean glandular trichome density values were compared between collection periods (seasons) and between leaf surfaces (adaxial and abaxial) by two-way analysis of variance (ANOVA), with comparisons of means by Tukey's test at a 5% significance level, using R 4.3.3 software (R Core Team, 2023). The chemical composition of the EOs was subjected to multivariate analysis using the MetaboAnalyst statistical platform.[36] The information about the samples (scores) was normalized, and the values of the variables (relative abundance) were autoscaled. The following methods were applied: principal component analysis (PCA); heat map construction based on the Euclidean distance; and debiased sparse partial correlation network modeling to identify positive and negative correlations between substances. A Pearson correlation analysis was also performed to assess relationships between antifungal activity and EO chemical composition using R 4.3.3 software (R Core Team, 2023).

3. RESULTS AND DISCUSSION

3.1. Glandular Trichome Density. Multicellular glandular trichomes were observed on the abaxial and adaxial surfaces of the leaves of *B. dracunculifolia*. The glandular trichomes were grouped in clusters located in depressions on both leaf surfaces (Figure 2), as reported in previous studies.[4,37]

The density of glandular trichomes for *B. dracunculifolia* showed no significant seasonal variation (F = 1.6623, p = 0.2053); however, there were differences between leaf surfaces. The adaxial surface had a higher density of glandular trichomes than the abaxial surface (F = 27.9775, p = 0.0000067), with the mean number of glandular trichomes being 142 and 74 in the dry season and 111 and 82 in the rainy season, respectively (Figure 3). Studies indicate that the intensity of light received by plant organs can positively modulate trichome development.[38,39] Thus, the greater density of glandular trichomes on the adaxial portion of the leaf blade of *B. dracunculifolia* could be related to the higher rates of solar radiation received by this side of the leaf. In this sense, the higher density of glandular trichomes on the adaxial side of the leaf blade could provide physical protection to the leaf by increasing the reflectance of solar radiation, which may limit water loss.[38] Furthermore, the secretion produced by the glandular trichomes of this species, which is predominantly composed of lipophilic substances,[3] could act in the chemical protection of the leaf surface. Hydrophobic exudates released on the plant surface play an important role in protecting vegetative organs against dehydration during the dry season, in addition to providing protection from herbivores and pathogens.[40,41] Considering that *B. dracunculifolia* is a pioneer species with rapid growth,[42] which typically occurs in open environments with high luminosity such as the Brazilian Pampa and Cerrado,[37] the abundance of trichomes that secrete lipid-based substances could be an important factor for plant performance under these environmental conditions.

3.2. Essential Oil Yield. Essential oil yield differed significantly between seasons (T = 4.0038; p = 0.0006531) with a higher mean yield in the rainy season. Percentage yield ranged from 0.38 to 0.74% (0.53 ± 0.10%) in the dry season and 0.46 to 0.97% (0.70 ± 0.16%) in the rainy season (Figure 4). Seasonal variation has a strong influence on specialized metabolite content, and climatic factors, such as temperature and rainfall, are commonly associated with these changes.[20] The present study recorded 38.95 mm of rainfall and 57.67% of soil saturation in the dry season (September). In the rainy season (March), the recorded rainfall was approximately 3.5 times higher, reaching 134.93 mm with 68.22% soil saturation. Total photosynthetically active radiation, a factor directly related to morphology, biomass production, and plant growth, was approximately 2835 W/m^2 in the dry season (September) and 3182 W/m^2 in the rainy season (March), for an increase of 12% between seasons.

Although the primary function of photosynthesis is the production of carbohydrates and other metabolites essential for cellular survival, it plays a central role in the synthesis of specialized metabolites as these derive from primary metabolites.[43] Thus, environmental factors that alter photosynthetic rates may influence the production of these substances indirectly[44,45] since their synthesis requires a significant amount of carbon.[46] Consequently, the concentrations of these compounds in plant tissues tend to vary seasonally and in response to diurnal cycles following climatic fluctuations.

The higher EO production observed for *B. dracunculifolia* during the rainy season is not correlated with trichome density, which did not vary between seasons but rather with environmental changes resulting from climatic factors. Shifts in temperature and precipitation regimes directly affect EO content and chemical composition.[47,48] In the studied population, for example, temperature peaks and higher rainfall levels coincided precisely with the rainy season (Figure 1), suggesting a direct relationship between these variables and EO production.

While *B. dracunculifolia* plants produced a higher quantity of EOs in the rainy season, the density of glandular trichomes in these plants was similar to that observed during the dry season. This increase in the yield, however, was not accompanied by structural changes in the secretory apparatus. Specialized secretory cells, such as those constituting EO producing trichomes, are characterized by possessing a very active biosynthetic machinery that can rapidly and efficiently transform imported sugar into EOs.[49] Considering that external factors can interact with cellular components, activating biochemical responses and leading to various changes in plant metabolism,[45] it is plausible that the environmental conditions during the rainy season enhanced the biochemical activity of the secretory cells in *B. dracunculifolia* glandular trichomes, consequently leading to increased EO production without a corresponding increase in the number of secretory structures.

3.3. Chemical Characterization of Essential Oils of *B. dracunculifolia*. Evaluation of the chemical composition of EOs by GC×GC−MS/FID resulted in the identification of 88 substances. Oxygenated sesquiterpenes were the most abundant group of substances (between 34.39 and 57.90%) for most individuals, with the exceptions being individuals 11, 12, and 13, with monoterpene hydrocarbons predominating in at least one collection period (Tables 2a and 2b). An additional table containing the mean values and standard deviations for each

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Table 2a. Chemical Composition (%) of Essential Oils from 15 Individuals of *B. dracunculifolia* Collected in the Dry Season, Obtained by Comprehensive Two-Dimensional Gas Chromatography (GC×GC−MS/FID)[a]

				Relative abundance (%)														
no.	compound	LRI exp.	LRI lit.	1Bd 01	1Bd 02	1Bd 03	1Bd 04	1Bd 05	1Bd 06	1Bd 07	1Bd 08	1Bd 09	1Bd 10	1Bd 11	1Bd 12	1Bd 13	1Bd 14	1Bd 15
	monoterpene hydrocarbons			12.58	19.90	17.33	16.94	23.45	16.23	20.72	19.76	27.00	29.15	41.98	27.81	27.21	12.15	23.06
1	*α*-thujene	928	924	0.10	0.39	0.03	0.57	0.34	0.08	0.33	0.26	0.05	0.72	0.41	0.03	0.36	-	0.49
2	***α*-pinene**	936	932	1.46	4.16	0.46	1.29	2.53	2.01	1.17	1.94	3.48	1.87	7.02	2.24	2.83	1.29	1.04
3	camphene	956	946	-	0.05	0.02	0.02	0.05	0.07	-	0.03	0.10	0.03	0.13	0.04	0.03	-	-
4	sabinene	978	969	-	-	-	0.11	0.19	0.11	0.14	0.07	0.18	0.15	0.13	-	-	0.09	0.03
5	***β*-pinene**	983	974	4.97	5.91	7.11	7.72	8.42	5.51	6.30	6.85	13.90	9.03	13.37	17.99	7.82	4.27	5.83
6	myrcene	992	989	0.99	2.51	0.35	0.77	0.97	0.51	1.19	0.70	1.45	1.38	2.17	1.71	1.55	1.02	1.55
8	*α*-phellandrene	1010	1002	0.03	-	-	-	0.06	0.02	-	-	0.02	0.04	0.03	0.02	-	-	0.06
9	*δ*-3-carene	1014	1008	0.11	-	0.18	-	0.12	0.13	0.32	0.19	0.24	0.04	-	0.02	0.18	0.04	0.07
10	*α*-terpinene	1021	1014	0.07	0.04	0.03	0.14	0.11	0.10	0.14	0.05	0.14	0.18	0.03	0.07	0.13	0.12	0.13
11	*p*-cymene	1029	1022	0.68	0.14	0.03	0.18	0.76	0.16	0.29	0.13	0.12	0.22	0.15	-	0.18	0.08	0.27
12	**limonene**	1033	1024	2.43	4.84	7.41	3.72	8.11	5.76	8.81	7.88	5.06	13.45	15.82	4.32	12.10	4.30	11.15
13	(*E*)-*β*-ocimene	1048	1044	0.45	0.69	0.73	0.87	0.60	0.90	0.71	0.64	0.91	0.61	0.86	0.46	0.68	0.65	0.52
15	*γ*-terpinene	1062	1054	0.21	0.20	0.19	0.42	0.26	0.14	0.17	0.20	0.19	0.40	0.68	0.22	0.41	0.19	0.50
17	terpinolene	1093	1086	0.35	0.38	0.17	0.56	0.31	0.11	0.22	0.19	0.15	0.45	0.57	0.17	0.43	0.10	0.57
19	(*E*)-4,8-dimethylnona-1,3,7-triene	1118	1113	0.73	0.59	0.62	0.57	0.62	0.62	0.93	0.63	1.01	0.58	0.61	0.52	0.51	-	0.85
	oxygenated monoterpenes			1.67	1.18	1.32	1.57	1.43	2.40	2.78	1.40	2.38	1.51	2.52	1.58	1.88	1.96	1.06
18	linalool	1104	1095	0.69	0.54	0.42	0.80	0.74	0.98	1.85	0.70	1.29	0.61	0.56	0.49	0.99	0.92	0.33
20	pinocarvone	1173	1160	-	-	-	-	-	-	-	-	-	-	0.05	-	-	-	-
21	terpinen-4-ol	1187	1174	0.43	0.36	0.21	0.32	0.29	0.73	0.24	0.17	0.36	0.32	0.61	0.38	0.34	0.40	0.35
22	*α*-terpineol	1202	1186	0.55	0.21	0.69	0.45	0.40	0.66	0.69	0.50	0.73	0.58	1.20	0.63	0.52	0.64	0.38
23	methyl citronellate	1261	1257	-	0.07	-	-	-	0.03	-	0.03	-	-	0.10	0.08	0.03	-	-
26	methyl nerolate	1280	1280	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	sesquiterpene hydrocarbons			16.19	19.28	11.35	17.73	16.30	13.57	13.96	19.64	25.48	15.12	10.49	19.02	18.05	16.68	19.47
27	*δ*-elemene	1347	1335	0.04	0.03	0.05	-	0.04	-	-	0.03	0.02	-	-	0.03	0.03	0.03	0.07
28	*α*-cubebene	1358	1348	0.04	0.05	0.05	0.06	0.10	0.06	0.06	0.04	0.06	-	0.07	0.03	0.12	0.08	0.10
30	*α*-ylangene	1384	1373	0.13	0.03	0.05	0.08	0.02	0.03	0.03	0.08	0.04	0.13	0.04	0.09	0.07	0.05	0.09
31	*α*-copaene	1388	1374	0.14	0.09	0.13	0.08	0.14	0.13	0.15	0.07	0.42	0.17	0.13	0.08	0.07	0.11	0.14
33	*β*-bourbonene	1393	1387	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
34	*β*-cubebene	1402	1389	-	0.07	0.09	0.04	0.03	0.07	0.03	0.03	0.02	0.02	0.02	-	-	-	-
35	*β*-elemene	1402	1389	0.26	0.15	-	0.07	0.17	0.19	0.20	0.10	0.27	0.19	0.15	0.24	0.11	0.18	0.22
36	*β*-longipinene	1410	1400	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
38	*α*-gurjunene	1424	1409	0.12	0.05	0.03	0.05	0.06	0.03	0.16	0.07	0.14	0.13	0.19	0.19	0.22	0.11	0.14
39	**(*E*)-*β*-caryophyllene**	1434	1417	1.88	2.67	1.29	4.50	1.35	1.24	1.40	1.85	3.53	1.29	1.29	1.33	1.56	1.45	2.48
40	*β*-copaene	1444	1430	0.10	0.06	0.05	0.06	0.35	0.12	0.17	0.18	0.26	0.10	0.07	0.15	0.13	0.20	0.11
41	*α*-guaiene	1444	1437	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
42	aromadendrene	1454	1439	0.59	0.43	0.71	0.10	0.57	0.39	0.50	1.07	1.64	0.79	0.85	0.97	1.70	1.24	0.96
44	guaia-6,9-diene	1461	1447	0.05	-	0.05	0.07	0.05	-	0.04	0.06	0.05	0.04	0.05	0.07	0.06	-	-
45	(*E*)-muurola-3,5-diene	1466	1451	0.10	0.09	0.04	0.06	0.14	0.10	0.10	0.05	0.17	0.11	0.07	0.06	0.03	0.09	0.08
46	*α*-humulene	1468	1452	0.36	0.63	0.71	1.17	0.60	0.83	0.63	1.07	0.64	-	0.73	0.60	0.66	0.47	0.75
47	*allo*-aromadendrene	1471	1458	0.05	-	-	0.02	-	-	0.07	-	0.13	0.72	0.07	0.13	-	-	-

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Table 2a. continued

no.	compound	LRI exp.	LRI lit.	Relative abundance (%) 1Bd 01	1Bd 02	1Bd 03	1Bd 04	1Bd 05	1Bd 06	1Bd 07	1Bd 08	1Bd 09	1Bd 10	1Bd 11	1Bd 12	1Bd 13	1Bd 14	1Bd 15
49	9-*epi*-(*E*)-caryophyllene	1478	1464	0.17	0.10	0.06	0.05	0.11	0.07	0.17	0.24	0.35	0.08	0.09	0.15	0.14	0.31	0.14
51	(*E*)-cadina-1(6),4-diene	1483	1475	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
52	*γ*-gurjunene	1488	1475	0.16	0.12	0.14	0.06	0.10	0.08	0.21	0.07	0.23	0.11	0.08	0.12	0.07	0.19	0.06
53	*γ*-muurolene	1490	1478	0.63	0.33	0.19	0.17	0.35	0.29	0.49	0.42	0.74	0.47	0.20	0.39	0.54	0.61	0.50
55	**germacrene D**	1498	1480	2.04	4.46	1.68	3.43	3.05	4.02	2.33	3.28	3.11	2.98	1.10	2.24	1.84	1.66	2.55
56	*β*-selinene	1503	1489	0.14	-	0.05	-	-	0.03	0.10	0.04	0.15	0.04	-	0.09	0.24	0.18	0.08
57	(*Z*)-*β*-guaiene	1500	1492	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
58	*α*-vetispirene	1505	1489	-	-	0.02	0.11	0.03	0.02	0.03	-	0.19	0.08	0.04	0.13	0.15	0.06	0.03
59	viridiflorene	1510	1496	0.28	0.28	0.55	0.34	0.57	0.30	0.17	1.26	1.25	0.52	0.70	1.04	0.81	0.96	0.61
60	**bicyclogermacrene**	1513	1500	4.22	6.61	3.34	4.30	4.50	3.17	3.12	6.46	8.10	3.70	1.88	8.66	5.57	4.75	7.12
61	*δ*-amorphene	1521	1511	0.16	0.11	0.26	0.18	0.20	0.28	0.11	0.21	0.20	0.14	0.13	0.19	0.10	0.18	0.20
62	*γ*-cadinene	1531	1513	0.65	0.37	0.28	0.52	0.59	0.25	0.46	0.43	0.50	0.47	0.24	0.03	0.09	0.06	0.53
63	***δ*-cadinene**	1536	1522	3.55	2.48	1.37	2.19	2.93	1.55	2.76	1.91	2.35	2.55	2.11	1.59	2.91	2.33	2.37
64	(*E*)-calamenene	1538	1528	0.08	0.05	0.03	-	-	0.02	0.05	-	0.18	-	-	-	0.20	0.08	0.05
65	*α*-cadinene	1554	1537	0.09	-	-	-	0.25	0.09	0.14	0.15	0.16	0.12	0.07	0.14	0.12	0.08	-
66	*α*-calacorene	1562	1544	0.12	0.02	0.13	0.03	-	-	0.06	0.23	0.43	0.05	0.12	0.40	0.28	0.98	0.09
68	*β*-calacorene	1582	1564	0.04	-	-	-	-	0.21	0.22	0.24	0.15	0.12	-	0.12	0.23	0.24	-
	oxygenated sesquiterpenes			56.78	45.35	52.74	53.48	48.04	56.11	50.97	44.03	28.70	41.70	35.67	36.59	35.17	51.27	44.31
43	cabreuva oxide A	1456	1444	0.11	0.13	0.08	0.62	0.13	0.22	0.20	0.15	0.13	0.23	0.17	0.13	-	0.29	0.07
48	cabreuva oxide B	1473	1462	1.70	1.40	1.23	1.12	1.12	1.54	1.35	0.42	1.32	1.44	0.64	1.05	0.54	2.14	1.01
50	cabreuva oxide C	1478	1466	0.10	0.10	0.06	0.09	-	0.10	0.11	-	0.09	0.14	-	0.04	0.07	0.24	0.11
54	cabreuva oxide D	1490	1479	1.20	0.94	1.02	0.96	0.68	1.05	0.94	0.44	0.81	0.97	0.48	0.78	0.39	1.51	0.89
67	**(*E*)-nerolidol**	1574	1561	36.72	29.11	33.50	35.55	34.08	39.45	33.66	32.09	14.40	27.38	23.23	13.38	15.89	24.96	29.26
69	maaliol	1582	1566	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
70	palustrol	1587	1567	0.53	0.42	0.92	0.12	0.25	0.20	0.73	0.36	0.88	0.26	0.29	0.77	0.87	0.38	0.40
71	**spathulenol**	1600	1577	5.07	5.23	7.96	6.17	5.26	6.76	8.28	5.72	3.75	6.93	7.12	13.35	9.15	17.30	6.41
72	caryophyllene oxide	1605	1582	0.41	-	-	1.62	0.43	0.43	-	0.40	-	-	0.46	-	-	0.17	0.50
73	globulol	1611	1590	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
74	cubeban-11-ol	1616	1601	0.18	0.16	0.02	0.61	-	0.74	0.77	0.23	0.32	0.08	0.10	1.07	0.22	0.36	0.14
75	salvial-4(14)-en-1-one	1611	1594	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
76	rosifoliol	1624	1600	0.26	0.29	0.97	0.08	-	0.23	0.17	0.27	2.00	0.13	0.14	0.22	0.23	0.54	0.34
77	1,10-di-*epi*-cubenol	1634	1618	0.12	0.19	0.06	0.14	0.19	0.22	0.36	0.10	0.24	0.24	0.08	0.23	-	0.18	0.08
78	viridiflorol	1618	1594	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
79	eremoligenol	1642	1629	0.32	0.35	0.19	0.07	-	0.21	0.14	0.40	0.49	0.24	0.21	0.36	0.17	0.29	0.15
80	epicubenol	1647	1627	0.53	0.10	0.64	0.28	0.30	0.33	0.30	0.35	0.28	-	0.50	0.07	0.66	0.26	0.29
81	muurola-4,10(14)-dien-1-*β*-ol	1642	1630	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
82	*t*-muurolol	1658	1640	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
83	cubenol	1653	1642	0.19	0.27	0.26	0.22	0.34	0.28	0.20	-	0.27	0.19	-	0.04	0.04	0.06	0.12
84	*α*-muurolol	1661	1644	3.60	2.02	1.79	2.57	1.54	0.38	0.43	0.78	2.42	1.03	0.25	1.51	2.47	0.67	1.68
85	***α*-cadinol**	1674	1652	5.62	3.61	2.40	2.89	3.24	1.63	2.73	1.83	0.44	1.64	1.36	2.97	3.33	0.57	2.44
86	isobicyclogermacrenal	1757	1733	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
87	germacra-4(15),5,10(14)-trien-1-*α*-ol	1711	1685	-	0.63	1.46	-	0.31	2.17	0.44	0.37	0.80	0.65	0.31	0.30	0.78	1.06	0.35

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Table 2a. continued

no.	compound		LRI exp.	LRI lit.	Relative abundance (%) 1Bd 01	1Bd 02	1Bd 03	1Bd 04	1Bd 05	1Bd 06	1Bd 07	1Bd 08	1Bd 09	1Bd 10	1Bd 11	1Bd 12	1Bd 13	1Bd 14	1Bd 15
88	eremophilone		1760	1734	0.12	0.40	0.18	0.37	0.17	0.17	0.16	0.12	0.06	0.15	0.33	0.32	0.36	0.29	0.07
		Others			1.04	0.60	1.11	0.70	1.01	0.95	0.96	1.71	1.28	0.61	1.53	0.54	1.65	1.31	1.41
7	2-pentyl-furan		992	990	-	-	-	-	-	0.02	-	0.03	-	-	0.03	-	0.02	-	0.04
14	phenylacetaldehyde		1055	1045	0.06	-	0.15	0.09	0.36	0.11	0.12	0.32	0.29	0.12	0.46	-	0.33	0.22	0.11
16	acetophenone		1079	1059	0.81	0.40	0.85	0.35	0.52	0.77	0.71	1.31	0.86	0.42	0.89	0.47	1.17	0.98	1.17
24	theaspirane A		1326	1299	-	-	0.03	-	-	-	-	-	-	0.02	0.03	-	0.09	-	-
25	3-(*E*)-hexenyl tiglate		1330	1315	0.06	0.12	-	0.14	0.03	-	0.08	-	-	-	0.09	-	-	-	-
29	ethyl hydrocinnamate		1360	1352	0.07	0.04	-	-	0.05	-	-	-	-	-	-	-	-	0.05	0.06
32	(*E*)-*β*-damascenone		1398	1383	0.04	0.04	0.02	0.07	0.02	0.05	0.03	0.05	0.10	0.03	0.03	0.07	0.04	0.06	0.03
37	methyl eugenol		1417	1403	-	-	0.06	0.05	0.03	-	0.02	-	0.03	0.02	-	-	-	-	-
	total identified				**88.26**	**86.31**	**83.85**	**90.43**	**90.23**	**89.26**	**89.39**	**86.54**	**84.84**	**88.09**	**92.19**	**85.78**	**83.96**	**83.37**	**89.31**

[a]LRI exp. = experimental linear retention index; LRI lit = linear retention index from literature;[33] **bold** = most abundant substances; (-) = not detected.

compound during the dry and rainy seasons, as well as across both periods, is provided as Supporting Information (Table S2).

With the exception of individual 12, (*E*)-nerolidol was the most abundant substance in all of the samples, with relative percentages ranging from 14.40 to 40.62% (mean 29.62 ± 8.86). For individual 12, *β*-pinene showed the highest concentration, with 17.99% and 28.68% in the dry and rainy seasons, respectively. Other substances found in high abundance were germacrene D (0.81 to 4.46; mean 2.46 ± 0.98%), bicyclogermacrene (0.81 to 10.88; 5.07 ± 2.49%), limonene (2.43 to 18.99; 8.30 ± 4.36%), spathulenol (3.75 to 17.3; 7.17 ± 3.44%), *α*-pinene (0.46 to 6.09; 2.66 ± 1.79%), *δ*-cadinene (1.37 to 3.98; 2.45 ± 0.64%), (*E*)-*β*-caryophyllene (0.85 to 4.58; 2.16 ± 1.25%), and *α*-cadinol (0.44 to 5.62%; 2.12 ± 1.16); the last was significantly more abundant in the dry season ($T = 2.1888$; $p = 0.04604$). Altogether, these ten major compounds accounted for 58.12 to 80.95% of the chemical composition of the EOs of this population (Tables 2a and 2b). Figure 5 illustrates the individual variability and chemical structures of these compounds.

The use of GC×GC offers an improved signal-to-noise ratio and peak resolution in the separation of the chromatographic peaks. This allows the characterization of compounds that would coelute in a one-dimensional GC analysis and enables the detection of compounds present in lower abundances. Figure 6 illustrates the higher resolving power of GC×GC compared to that of GC and shows an example of overlapping peaks in GC that were resolved in the second dimension of GC×GC. The substances *β*-elemene and aromadendrene, which appear as a single peak in GC, were separated into two distinct peaks in GC×GC, making it possible to identify the substances as *β*-cubebene and cabreuva oxide A (with the latter being especially valued by the fragrance industry[12]). This demonstrated increased resolution and signal clarity allows reliable identification even of minor compounds.

Differences between seasons were mainly driven by compounds present at low abundances and exclusive to each collection period. The following compounds were identified in the dry season: (*E*)-*β*-damascenone, *α*-vetispirene, *β*-calacorene, 1,10-di-*epi*-cubenol, eremoligenol, germacra-4(15),5,10(14)-trien-1-*α*-ol, eremophilone, 2-pentyl-furan, pinocarvone, methyl citronellate, theaspirane A, 3-(*E*)-hexenyl tiglate, and ethyl hydrocinnamate. In contrast, methyl nerolate, *β*-bourburene, *α*-guaiene, (*E*)-cadine-1(6),4-diene, (*Z*)-*β*-guaiene, maaliol, globulol, salvial-4(14)-en-1-one, viridiflorol, muurola-4,10(14)-dien-1-*β*-ol, *γ*-muurolol, *β*-longipinene, and isobicyclogermacrenal were characteristic of the rainy season. These compositional differences were corroborated by a principal component analysis (PCA).

Principal component analysis (PCA) of the chemical composition data revealed two principal components (PC1 and PC2), which together explained 29.57% of the total variation. The seasons were divided into two clusters. To complement the PCA, partial least-squares discriminant analysis (PLS-DA) was performed and accounted for 25.4% of the total variation in the data. The variable importance in projection (VIP) scores for the ten main features contributing to group separation were viridiflorol, muurola-4,10(14)-dien-1-*β*-ol, isobicyclogermacrene, *α*-cubebene, *γ*-muurolol, and globulol during the rainy season and (*E*)-*β*-damascenone, 1,10-di-*epi*-cubenol, eremoligenol, and eremophilone during the dry season (Figure 7).

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Table 2b. Chemical Composition (%) of Essential Oils from 15 Individuals of *B. dracunculifolia* Collected in the Rainy Season, Obtained by Comprehensive Two-Dimensional Gas Chromatography (GC×GC−MS/FID)[a]

				Relative abundance (%)														
no.	compound	LRI exp.	LRI lit.	2Bd 01	2Bd 02	2Bd 03	2Bd 04	2Bd 05	2Bd 06	2Bd 07	2Bd 08	2Bd 09	2Bd 10	2Bd 11	2Bd 12	2Bd 13	2Bd 14	2Bd 15
	monoterpene hydrocarbons			21.71	21.94	23.44	16.62	22.24	31.64	18.17	22.71	22.26	22.42	43.07	45.82	45.24	29.43	15.23
1	α-thujene	928	924	0.45	0.36	0.02	0.22	0.36	0.14	0.49	0.25	0.03	0.60	0.39	0.09	0.59	0.04	0.50
2	**α-pinene**	936	932	3.85	4.39	0.53	0.77	2.71	2.99	1.27	1.86	2.32	1.23	6.09	5.49	5.96	4.63	0.96
3	camphene	956	946	0.09	0.03	0.06	-	0.05	0.08	0.05	0.06	0.04	0.03	0.05	0.14	0.09	0.10	-
4	sabinene	978	969	0.17	0.39	-	0.16	0.19	0.14	0.15	-	-	0.07	0.53	-	0.33	-	0.09
5	**β-pinene**	983	974	8.67	6.39	11.59	10.03	8.37	9.74	3.78	9.60	12.28	5.54	13.85	28.68	14.10	11.62	2.92
6	myrcene	992	989	1.39	2.94	0.50	0.87	1.15	1.47	0.85	1.47	1.33	1.05	2.85	2.49	2.82	2.73	0.72
8	α-phellandrene	1010	1002	-	0.02	-	-	-	0.04	-	0.02	-	0.03	0.05	-	0.04	0.04	-
9	δ-3-carene	1014	1008	0.19	-	0.19	-	0.08	0.23	0.37	0.18	0.27	0.03	-	0.05	0.10	0.13	0.05
10	α-terpinene	1021	1014	0.19	0.13	-	0.09	0.11	0.23	0.13	0.08	0.08	0.09	0.17	0.17	0.21	0.12	0.11
11	*p*-cymene	1029	1022	0.05	0.22	-	0.12	0.08	0.25	0.16	0.14	-	-	0.11	-	0.19	-	0.07
12	**limonene**	1033	1024	4.68	5.31	9.27	2.43	7.56	14.03	8.97	7.03	3.87	11.86	17.49	6.72	18.99	7.30	8.34
13	(*E*)-β-ocimene	1048	1044	0.73	0.47	0.54	0.52	0.43	0.88	0.83	0.60	1.10	0.55	0.41	0.87	0.61	1.68	0.36
15	γ-terpinene	1062	1054	0.30	0.36	0.12	0.38	0.21	0.29	0.29	0.33	0.30	0.42	0.38	0.44	0.38	0.28	0.26
17	terpinolene	1093	1086	0.34	0.34	0.16	0.33	0.30	0.16	0.20	0.36	0.21	0.52	0.29	0.31	0.42	0.18	0.37
19	(*E*)-4,8-dimethylnona-1,3,7-triene	1118	1113	0.61	0.59	0.46	0.70	0.64	0.97	0.63	0.73	0.43	0.40	0.41	0.37	0.41	0.58	0.48
	oxygenated monoterpenes			1.07	0.61	0.86	0.46	0.72	3.25	1.70	1.24	2.23	0.99	1.21	1.21	2.18	1.60	0.32
18	linalool	1104	1095	0.55	0.38	0.26	0.23	0.21	1.82	0.90	0.36	0.99	0.29	0.29	0.23	1.18	0.59	0.12
20	pinocarvone	1173	1160	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
21	terpinen-4-ol	1187	1174	0.13	0.16	0.21	0.14	0.19	0.67	0.12	0.34	0.39	0.23	0.51	0.46	0.36	0.47	0.09
22	α-terpineol	1202	1186	0.39	0.07	0.39	0.09	0.32	0.76	0.68	0.54	0.79	0.47	0.41	0.52	0.64	0.54	0.11
23	methyl citronellate	1261	1257	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
26	methyl nerolate	1280	1280	-	-	-	-	-	-	-	-	0.06	-	-	-	-	-	-
	sesquiterpene hydrocarbons			16.16	16.17	13.86	26.47	19.65	16.19	16.85	22.57	17.48	10.13	11.12	25.83	12.26	22.89	28.50
27	δ-elemene	1347	1335	0.06	0.04	0.05	0.07	0.15	0.10	0.08	0.07	0.12	0.06	0.04	0.11	0.07	0.10	0.16
28	α-cubebene	1358	1348	0.08	0.24	0.13	0.24	0.23	0.22	0.10	0.15	0.15	0.10	0.13	0.13	0.15	0.19	0.20
30	α-ylangene	1384	1373	0.05	0.03	0.05	0.05	0.05	0.05	-	0.11	0.05	0.06	0.04	0.10	0.04	0.10	-
31	α-copaene	1388	1374	0.11	0.21	0.21	0.33	0.15	0.27	0.25	0.20	0.32	0.09	0.10	0.24	0.03	0.25	0.35
33	β-bourbonene	1393	1387	-	0.05	0.11	0.05	0.07	0.02	0.11	0.01	0.07	0.03	0.01	0.13	-	0.07	0.07
34	β-cubebene	1402	1389	-	0.07	-	0.02	-	0.01	-	0.05	-	-	-	-	-	-	-
35	β-elemene	1402	1389	0.23	0.24	0.32	0.53	0.23	0.30	0.22	0.47	0.36	0.14	0.08	0.45	0.17	0.37	0.44
36	β-longipinene	1410	1400	-	-	-	-	-	-	-	-	-	-	-	0.02	-	0.21	-
38	α-gurjunene	1424	1409	0.12	0.05	0.18	0.10	0.11	0.07	0.12	0.28	0.22	0.07	0.07	0.30	0.12	0.24	0.17
39	**(*E*)-β-caryophyllene**	1434	1417	1.96	1.99	1.56	6.74	2.45	1.84	2.14	2.59	2.63	0.93	1.40	2.20	0.85	1.90	4.58
40	β-copaene	1444	1430	0.07	0.12	0.23	0.33	0.16	0.25	0.15	0.32	0.29	0.09	0.11	0.39	0.14	0.31	0.21
41	α-guaiene	1444	1437	-	-	-	-	-	-	-	-	-	-	0.06	0.03	0.06	-	-
42	aromadendrene	1454	1439	0.52	0.62	1.01	1.17	0.92	0.95	0.90	1.79	1.52	0.62	1.00	2.54	1.01	1.43	1.51
44	guaia-6,9-diene	1461	1447	0.05	0.05	0.05	-	0.04	-	-	-	-	-	-	-	-	-	-
45	(*E*)-muurola-3,5-diene	1466	1451	0.06	0.09	0.07	0.06	0.05	0.07	0.17	0.06	0.06	0.02	0.07	0.07	0.08	0.08	0.12
46	α-humulene	1468	1452	0.91	0.54	0.45	0.94	0.69	0.86	0.90	0.68	0.62	0.43	0.75	0.75	0.40	1.87	1.04
47	*allo*-aromadendrene	1471	1458	0.03	-	-	0.09	0.14	0.33	0.04	-	0.12	0.05	0.05	0.28	0.07	-	0.11

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Table 2b. continued

no.	compound	LRI exp.	LRI lit.	Relative abundance (%) 2Bd 01	2Bd 02	2Bd 03	2Bd 04	2Bd 05	2Bd 06	2Bd 07	2Bd 08	2Bd 09	2Bd 10	2Bd 11	2Bd 12	2Bd 13	2Bd 14	2Bd 15
49	9-epi-(*E*)-caryophyllene	1478	1464	0.05	0.25	0.13	0.14	-	0.33	-	0.30	0.15	-	0.12	0.13	-	-	-
51	(*E*)-cadina-1(6),4-diene	1483	1475	-	0.13	0.05	0.07	-	-	-	0.06	-	0.01	0.08	-	-	-	-
52	*γ*-gurjunene	1488	1475	-	-	-	-	0.08	-	-	-	0.10	-	0.32	-	-	0.10	-
53	*γ*-muurolene	1490	1478	0.34	0.49	0.31	0.59	0.30	0.79	0.60	0.67	0.51	0.30	-	0.79	0.23	0.80	0.42
55	**germacrene D**	1498	1480	2.20	3.22	1.61	4.40	3.02	2.75	1.95	2.20	0.81	1.08	1.59	2.04	0.87	2.77	3.51
56	*β*-selinene	1503	1489	3.87	-	-	0.10	0.18	0.09	0.24	0.30	0.14	-	-	0.32	0.06	0.16	0.20
57	(*Z*)-*β*-guaiene	1500	1492	-	0.06	-	0.13	0.18	0.06	-	0.18	0.18	-	0.11	0.19	0.02	0.06	-
58	*α*-vetispirene	1505	1489	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
59	viridiflorene	1510	1496	-	-	0.77	0.68	-	0.42	0.54	2.33	-	-	0.71	1.95	-	-	0.11
60	**bicyclogermacrene**	1513	1500	-	4.12	4.08	6.36	6.44	3.56	3.10	6.78	6.43	2.85	0.81	9.28	4.18	7.65	10.88
61	*δ*-amorphene	1521	1511	0.21	0.27	0.18	0.05	0.13	0.23	0.30	0.11	0.18	0.21	0.13	0.11	0.12	0.21	0.18
62	*γ*-cadinene	1531	1513	0.95	0.62	0.24	0.73	0.89	0.37	1.00	0.57	0.57	0.48	0.60	0.67	0.62	0.80	0.86
63	***δ*-cadinene**	1536	1522	3.98	2.51	1.56	2.37	2.77	2.00	3.63	2.23	1.74	1.86	2.53	2.25	2.79	3.16	3.19
64	(*E*)-calamenene	1538	1528	-	-	-	-	0.02	-	-	-	0.14	-	-	-	-	-	-
65	*α*-cadinene	1554	1537	0.26	0.13	0.40	0.13	0.16	0.25	0.24	0.06	-	0.65	0.14	0.36	0.12	-	0.13
66	*α*-calacorene	1562	1544	0.05	0.03	0.11	-	0.04	-	0.07	-	-	-	0.07	-	0.06	0.06	0.06
68	*β*-calacorene	1582	1564	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	oxygenated sesquiterpenes			54.09	53.83	55.22	51.99	52.82	41.98	56.74	47.27	46.76	58.80	38.91	20.96	31.53	35.74	52.03
43	cabreuva oxide A	1456	1444	-	0.19	0.10	0.12	0.17	0.34	-	-	-	0.16	-	-	-	0.35	-
48	cabreuva oxide B	1473	1462	0.58	1.00	0.96	0.52	0.41	1.44	0.85	0.54	0.64	1.27	0.25	0.72	0.47	0.17	0.36
50	cabreuva oxide C	1478	1466	0.17	0.08	0.05	0.04	-	-	0.25	0.04	0.04	-	0.12	-	0.16	-	-
54	cabreuva oxide D	1490	1479	0.60	0.61	0.80	0.38	0.42	0.51	0.64	0.47	0.66	0.90	0.24	0.58	0.25	1.35	0.27
67	**(*E*)-nerolidol**	1574	1561	36.59	35.15	34.15	35.59	40.27	28.02	40.61	30.53	22.91	40.62	25.99	7.55	18.33	29.06	40.43
69	maaliol	1582	1566	0.19	0.66	0.22	0.24	0.18	0.19	-	0.22	0.39	-	0.08	-	0.78	-	0.10
70	palustrol	1587	1567	0.46	0.22	0.34	0.47	0.31	0.27	0.41	0.50	0.81	0.25	0.25	0.65	0.31	0.32	0.23
71	**spathulenol**	1600	1577	5.00	8.95	12.24	7.37	5.97	7.64	8.03	6.99	11.96	7.45	7.40	7.09	-	-	4.53
72	caryophyllene oxide	1605	1582	0.65	0.41	0.22	0.32	0.37	0.24	0.60	0.31	0.58	0.21	0.20	0.41	0.44	-	0.57
73	globulol	1611	1590	0.69	1.55	1.88	0.52	0.56	0.47	0.26	2.13	2.35	2.49	1.26	1.34	2.58	-	1.89
74	cubeban-11-ol	1616	1601	-	-	-	0.10	-	-	0.15	0.11	0.24	-	-	0.07	0.14	0.11	0.07
75	salvial-4(14)-en-1-one	1611	1594	0.05	-	0.06	0.13	-	0.09	-	-	-	0.07	-	0.03	-	0.04	0.04
76	rosifoliol	1624	1600	0.08	0.05	0.14	0.23	0.17	0.21	0.42	0.32	0.07	0.36	0.13	0.06	0.24	0.38	0.12
77	1,10-di-*epi*-cubenol	1634	1618	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
78	viridiflorol	1618	1594	0.70	0.45	0.60	0.73	0.56	0.35	0.58	0.82	1.15	0.54	0.57	1.03	1.51	0.28	0.40
79	eremoligenol	1642	1629	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
80	epicubenol	1647	1627	0.55	0.38	0.34	0.18	0.11	0.17	0.27	0.27	0.23	0.04	0.04	0.25	0.19	0.14	0.05
81	muurola-4,10(14)-dien-1-*β*-ol	1642	1630	0.08	0.07	0.29	0.17	0.24	0.19	0.16	0.30	-	0.14	0.30	0.04	0.23	0.20	0.28
82	*t*-muurolol	1658	1640	2.90	-	0.47	1.84	1.21	0.58	1.35	1.35	1.35	1.28	0.72	0.42	0.61	1.01	0.41
83	cubenol	1653	1642	0.41	0.30	0.31	0.25	0.27	0.27	-	0.38	0.30	0.53	0.29	-	1.68	0.49	0.72
84	*α*-muurolol	1661	1644	-	1.11	0.58	0.41	-	0.17	0.31	0.15	-	0.34	0.13	-	0.28	0.22	0.08
85	***α*-cadinol**	1674	1652	3.89	2.20	1.13	2.28	1.39	0.73	1.53	1.76	2.72	1.89	0.83	0.62	3.06	1.42	1.33
86	isobicyclogermacrenal	1757	1733	0.50	0.45	0.34	0.10	0.21	0.10	0.32	0.08	0.36	0.26	0.11	0.10	0.27	0.20	0.15
87	germacra-4(15),5,10(14)-trien-1-*α*-ol	1711	1685	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

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Table 2b. continued

no.	compound	LRI exp.	LRI lit.	Relative abundance (%)														
				2Bd 01	2Bd 02	2Bd 03	2Bd 04	2Bd 05	2Bd 06	2Bd 07	2Bd 08	2Bd 09	2Bd 10	2Bd 11	2Bd 12	2Bd 13	2Bd 14	2Bd 15
88	eremophilone	1760	1734	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Others			0.48	0.55	0.41	0.07	0.39	1.51	0.74	0.81	0.46	0.45	0.35	0.31	0.8	0.92	0.32
7	2-pentyl-furan	992	990	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
14	phenylacetaldehyde	1055	1045	0.14	0.22	-	-	0.10	0.23	0.09	0.16	-	0.20	0.13	-	0.40	0.13	0.04
16	acetophenone	1079	1059	0.34	0.33	0.41	0.07	0.29	1.28	0.65	0.65	0.40	0.25	0.22	0.31	0.40	0.79	0.28
24	theaspirane A	1326	1299	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
25	3-(*E*)-hexenyl tiglate	1330	1315	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
29	ethyl hydrocinnamate	1360	1352	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
32	(*E*)-*β*-damascenone	1398	1383	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
37	methyl eugenol	1417	1403	-	-	-	-	-	-	-	-	0.06	-	-	-	-	-	-
	total identified			**93.51**	**93.10**	**93.79**	**95.61**	**95.82**	**94.57**	**94.20**	**94.60**	**89.19**	**92.79**	**94.66**	**94.13**	**92.01**	**90.58**	**96.40**

[a]LRI exp. = experimental linear retention index; LRI lit = linear retention index from literature;[33] **bold** = most abundant substances; (-) = not detected.

The debiased sparse partial correlation (DSPC) network revealed both positive and negative correlations between compounds, which may suggest potential biosynthetic relationships in *B. dracunculifolia*. For instance, *α*-pinene exhibited strong negative correlations with myrcene and linalool. These compounds share geranyl pyrophosphate (GPP) as a common precursor, suggesting that the negative correlations may reflect competitive or divergent regulation along the same biosynthetic pathway.[50,51] Similarly, a negative correlation was observed between sabinene and terpinolene. In contrast, a strong positive correlation was detected between (*E*)-nerolidol and *p*-cymene, which may indicate coregulation in their biosynthesis or a shared response to environmental factors (Figure 8). The complete correlation matrix is available in the Supporting Information (Table S1).

The heatmap showed the formation of two well-defined clusters. One comprised all individuals from the dry season and was highly correlated with the substances (*E*)-*β*-damascenone, *α*-vetispirene, *β*-calacorene, 1,10-di-*epi*-cubenol, *α*-muurolol, eremoligenol, germacra-4(15),5,10(14)-trien-1-*α*-ol, and eremophilone, The other included all individuals from the rainy season, and was highly correlated with *β*-bourburene, (*Z*)-*β*-guaiene, maaliol, globulol, viridiflorol, muurola-4,10(14)-dien-1-*β*-ol, *γ*-cadinene, *β*-elemene, *δ*-elemene, *α*-cubebene, *γ*-muurolol, and isobicyclogermacrenal (Figure 9). These findings corroborate the active role that climatic variation plays in shaping the volatile composition of *B. dracunculifolia* EOs.

3.4. Antifungal Activity of *B. dracunculifolia* Essential Oils against *A. nomius*, *F. graminearum* and *F. verticillioides*.

The EOs of individuals from the *B. dracunculifolia* population showed mycelial growth inhibitory activities for all of the fungi studied, compared to the control. The highest inhibition rates for *A. nomius* were reached with EO from individual 1BD02, which were 25.30% in the first (72 h after inoculation) and 10.69% in the second reading (144 h after inoculation). The highest inhibition rates for *F. graminearum* were reached with EO from individual 1BD06, reaching 40.24% in the first reading and 12.55% in the second reading. Lastly, the highest inhibition rates for *F. verticillioides* were reached with the EO of individual 1BD02, reaching 24.42% in the first reading and 22.76% in the second reading (Figure 10). These findings are in line with previous reports, with significant inhibition by *B. dracunculifolia* EOs at concentrations of 3000 mg·L^{-1} against *Fusarium oxysporum* and *Fusarium solani*.[13] In addition, antifungal activity against species of *Aspergillus* has also been observed.[10] These results reinforce the selection of genotypes 1BD02 and 1BD06 as promising candidates for the commercial development of antifungal formulations based on their EOs.

Paired *t* tests performed for each of the studied fungi to determine whether season influenced the antimicrobial activity of the EOs found no significant differences between seasons in the measurements made for the fungi *A. nomius* and *F. graminearum*. The EOs showed higher inhibitory potential against *F. verticillioides* in the dry season, in both the first and second measurements ($t = 1.9921$; $p = 0.02918$ and $F = 1.9714$; $p = 0.02991$, respectively), demonstrating seasonal variability in the EOs.

Based on the observed variations and to understand which substances contributed the most to mycelial growth inhibition, a Pearson correlation analysis was performed between EO compounds and percentage of fungal inhibition. The substances *p*-cymene ($r = 0.79$), *α*-cadinol ($r = 0.82$), and *γ*-muurolene ($r = 0.87$) exhibited the highest positive correlations with EO

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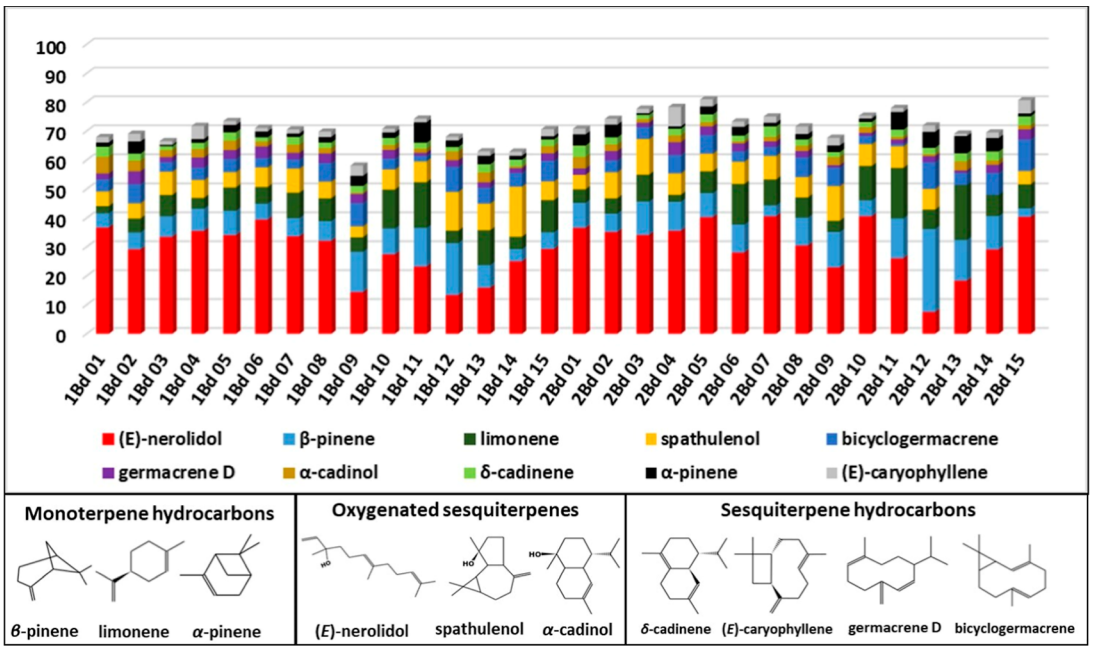

Figure 5. Relative proportions (%) and chemical structures of the 10 major compounds identified in the essential oils of *B. dracunculifolia* in the dry (1Bd) and rainy (2Bd) seasons.

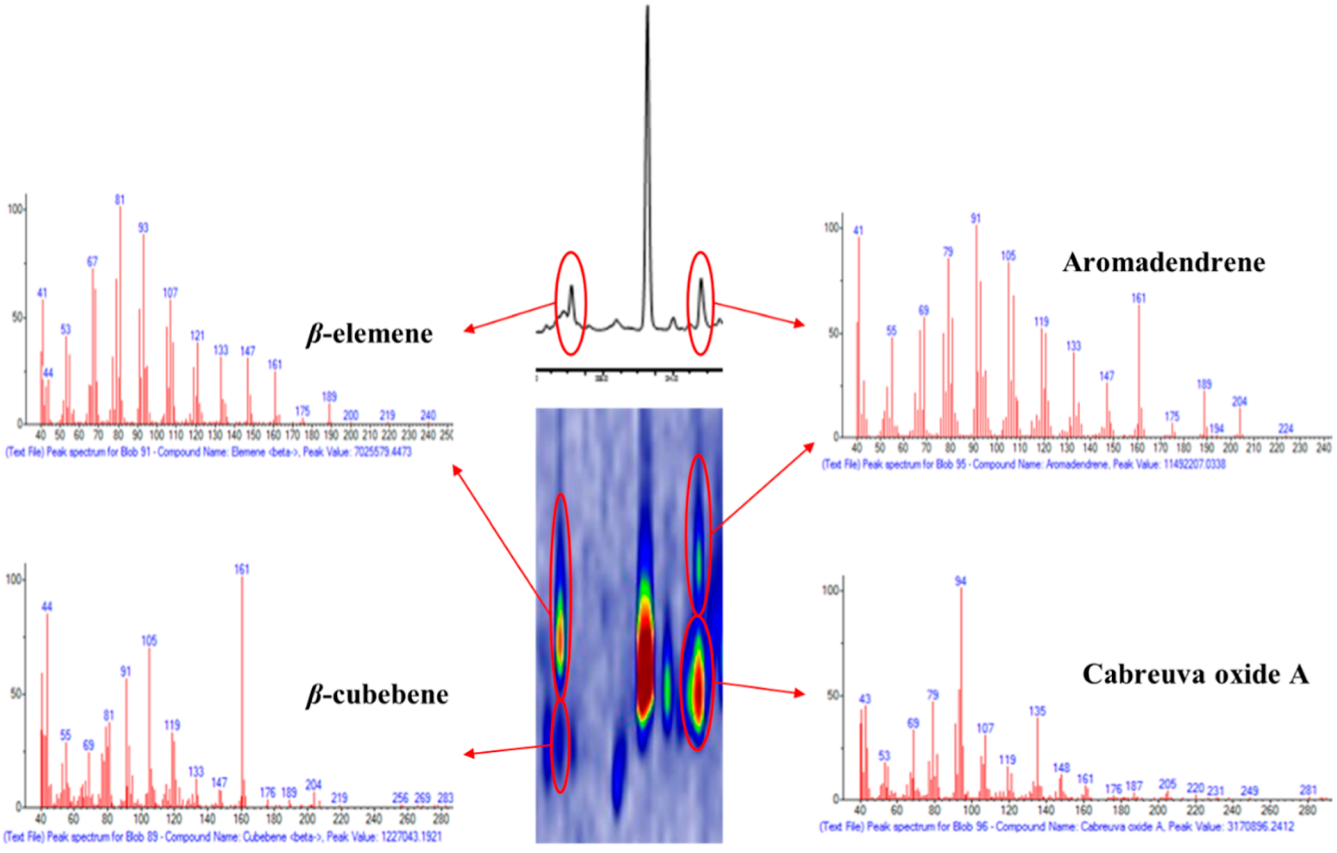

Figure 6. Diagram illustrating the coelution of the substances *β*-elemene with *β*-cubebene and aromadendrene with cabreuva oxide A.

antifungal activity, suggesting that they may be associated with its inhibitory effect on the evaluated fungi. According to the software PASS (Prediction of Activity Spectra for Substances),[52] which can predict molecular effects in silico, with indices ranging from 0 to 1, *p*-cymene, *α*-cadinol, and *γ*-muurolene have a probability of 0.368, 0.454, and 0.489, respectively, of being active against fungi. The strong correlation observed for *p*-cymene is consistent with previously described mechanisms, such as destabilization of membrane integrity, impairment of ATP synthesis, and induction of oxidative stress; *p*-cymene also acts as a synergistic agent for other substances.[53] The high antifungal efficacy of *α*-cadinol has also been reported,[54]

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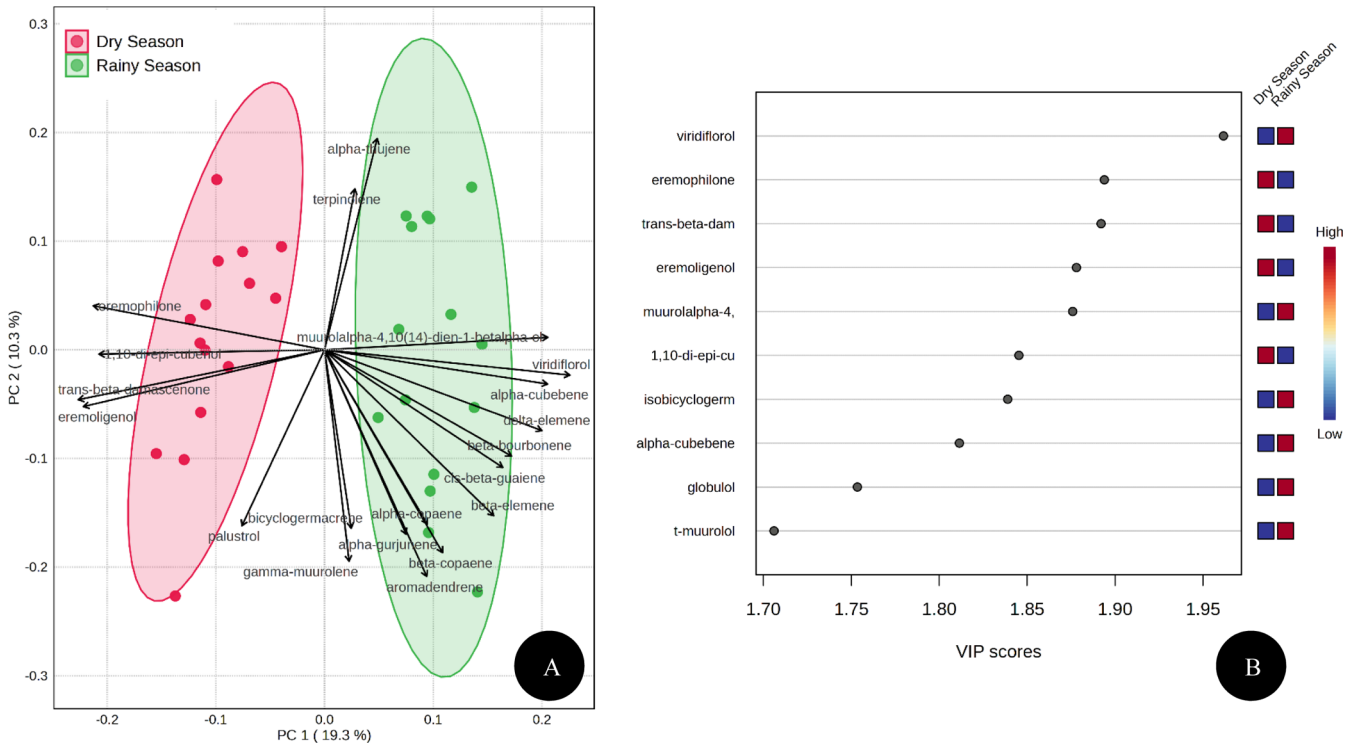

Figure 7. (A) Biplot of principal component analysis (PCA; showing the 20 variables with the highest loading scores) and (B) variable importance in projection (VIP) from partial least-squares discriminant analysis (PLS-DA; showing the 10 main features) of the chemical composition of *B. dracunculifolia* EOs in two collection periods (dry and rainy seasons).

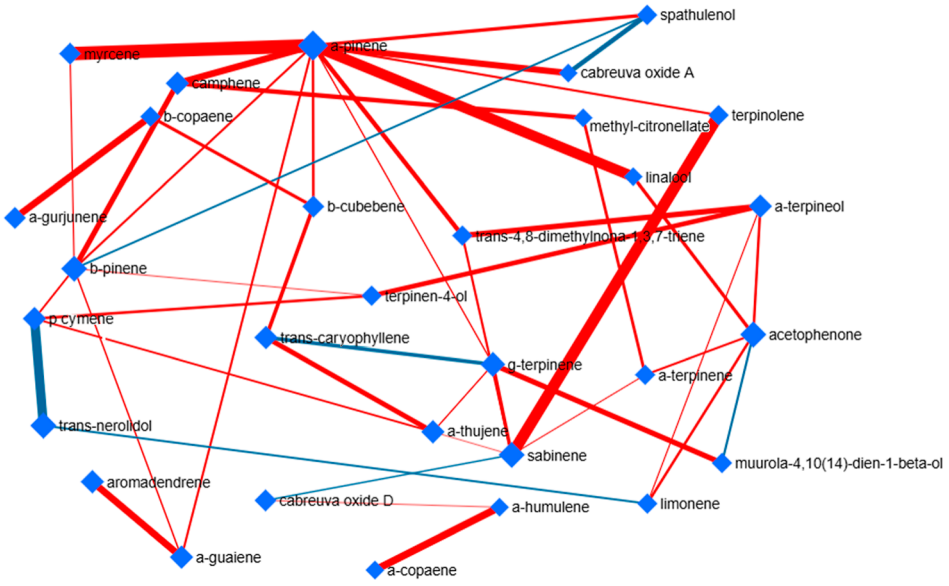

Figure 8. DSPC network demonstrating the main positive and negative correlations between the compounds present in *B. dracunculifolia* EOs.

showing strong inhibitory activity against fungi such as *Rhizoctonia solani*, *F. oxysporum*, *Colletotrichum gloeosporioides*, and *Ganoderma australe*, with half-maximal inhibitory concentration (IC_{50}) values ranging from 11.7 to 44.3 $\mu g \cdot mL^{-1}$, which is higher than those of other sesquiterpenes and monoterpenes. Further antifungal activity of EOs containing high levels of γ-muurolene was observed for the plants *Eupatorium adenophorum* and *Cryptomeria japonica*, corroborating the results of the present study.[55,56]

Major constituents, such as (*E*)-β-caryophyllene, limonene, (*E*)-nerolidol, spathulenol, and α- and β-pinene, also have antifungal properties recorded in the literature.[57−60] The synergistic effect between the substances contained in the EOs and their percentages acts on multiple sites of action of the microorganism, causing membrane destruction, increased permeability, oxidative stress, inhibition of ergosterol (essential for the cell membrane of these organisms), and alteration of cytoplasm composition, among others effects. The different percentages of substances in each genotype led to intra-population variability in the antifungal activity of the different strains.[61] These findings reinforce the potential of *B. dracunculifolia* EOs as natural alternatives to synthetic fungicides

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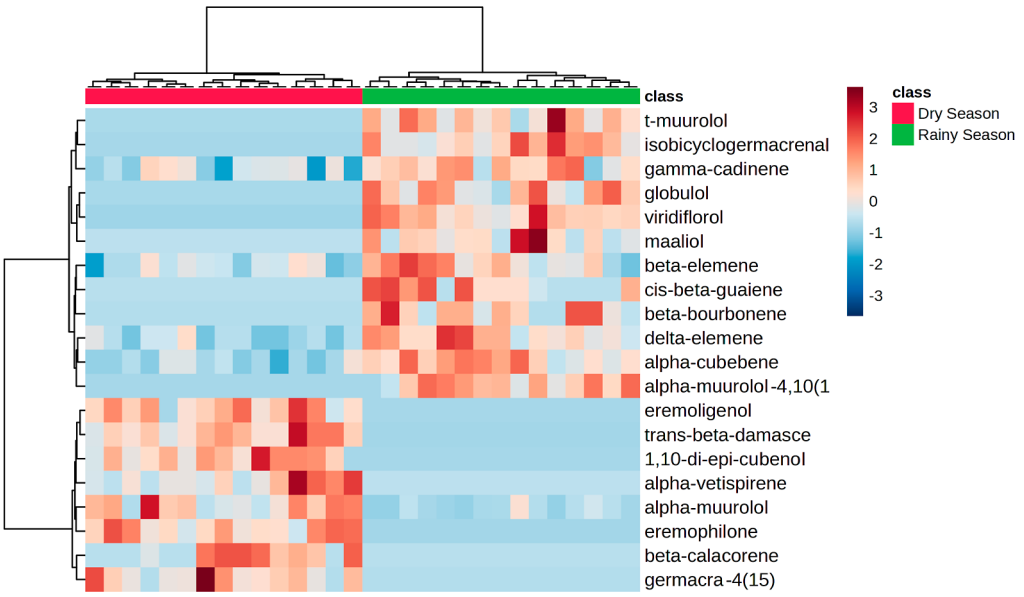

Figure 9. Heatmap based on the 20 chemical substances responsible for the separation of essential oils of *B. dracunculifolia* in the dry and rainy seasons.

for protecting crops and stored grains against toxigenic fungi, particularly in sustainable agricultural systems.

In summary, *B. dracunculifolia* exhibited a higher density of glandular trichomes on the adaxial leaf surface, with no seasonal variation in trichome density. However, essential oil yields were significantly higher during the rainy season, suggesting increased accumulation in trichomes under these conditions. Chemical composition varied seasonally, with GC×GC analysis resolving coeluting compounds and identifying low-abundance substances critical for distinguishing between seasons.

The essential oils of *B. dracunculifolia* demonstrated antifungal activity against *A. nomius*, *F. graminearum*, and *F. verticillioides*, underscoring its potential as a natural alternative to synthetic fungicides for crop and stored grain protection. Its inhibitory efficacy varied according to seasonal and intrapopulational chemical variability. Remarkably, minor compounds such as *p*-cymene, α-cadinol, and γ-muurolene showed the strongest correlation with antifungal activity, suggesting a possible association. The most successful genotypes for antifungal activity were 1BD02 (effective against *A. nomius* and *F. verticillioides*) and 1BD06 (active against *F. graminearum*), both of which were obtained in the dry season. These findings establish a predictive framework for genotype selection and harvest timing to maximize the yield and biological activity of bioactive compounds from essential oil, offering actionable insights for the pharmaceutical, agriculture, and cosmetic sectors.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsagscitech.5c00267.

Table of correlation values among compounds identified in the essential oils of *B. dracunculifolia* (Table S1); Means and standard deviations of compounds of *B. dracunculifolia* (Table S2) (PDF)

■ AUTHOR INFORMATION

Corresponding Author

Marcia O. M. Marques − *Plant Genetic Resources Center, Agronomic Institute (IAC), Campinas 13020-902 São Paulo, Brazil;* orcid.org/0000-0001-8270-4308; Email: marcia.marques@sp.gov.br

Authors

Paulo H. Furlan − *Institute of Biosciences, São Paulo State University (Unesp), Botucatu 18618-970 São Paulo, Brazil; Plant Genetic Resources Center, Agronomic Institute (IAC), Campinas 13020-902 São Paulo, Brazil;* orcid.org/0000-0002-0449-2466

Júlio C. R. L. Silva − *Plant Genetic Resources Center, Agronomic Institute (IAC), Campinas 13020-902 São Paulo, Brazil;* orcid.org/0000-0003-0120-2424

Roselaine Facanali − *Plant Genetic Resources Center, Agronomic Institute (IAC), Campinas 13020-902 São Paulo, Brazil*

Daniela M. Soares − *Plant Genetic Resources Center, Agronomic Institute (IAC), Campinas 13020-902 São Paulo, Brazil*

Mariana N. F. Cabral − *Plant Genetic Resources Center, Agronomic Institute (IAC), Campinas 13020-902 São Paulo, Brazil; School of Agriculture, São Paulo State University (Unesp), Botucatu 18610-034 São Paulo, Brazil;* orcid.org/0000-0002-1210-817X

Tatiane M. Rodrigues − *Institute of Biosciences, São Paulo State University (Unesp), Botucatu 18618-970 São Paulo, Brazil*

Aline R. Martins − *Department of Biology and Zootechny, São Paulo State University (Unesp), Ilha Solteira 15385-000 São Paulo, Brazil*

Eduardo M. da Gloria − *Luiz de Queiroz College of Agriculture (ESALQ-USP), Department of Biological Sciences, University of São Paulo, Piracicaba 13418-900 São Paulo, Brazil*

Complete contact information is available at: https://pubs.acs.org/10.1021/acsagscitech.5c00267

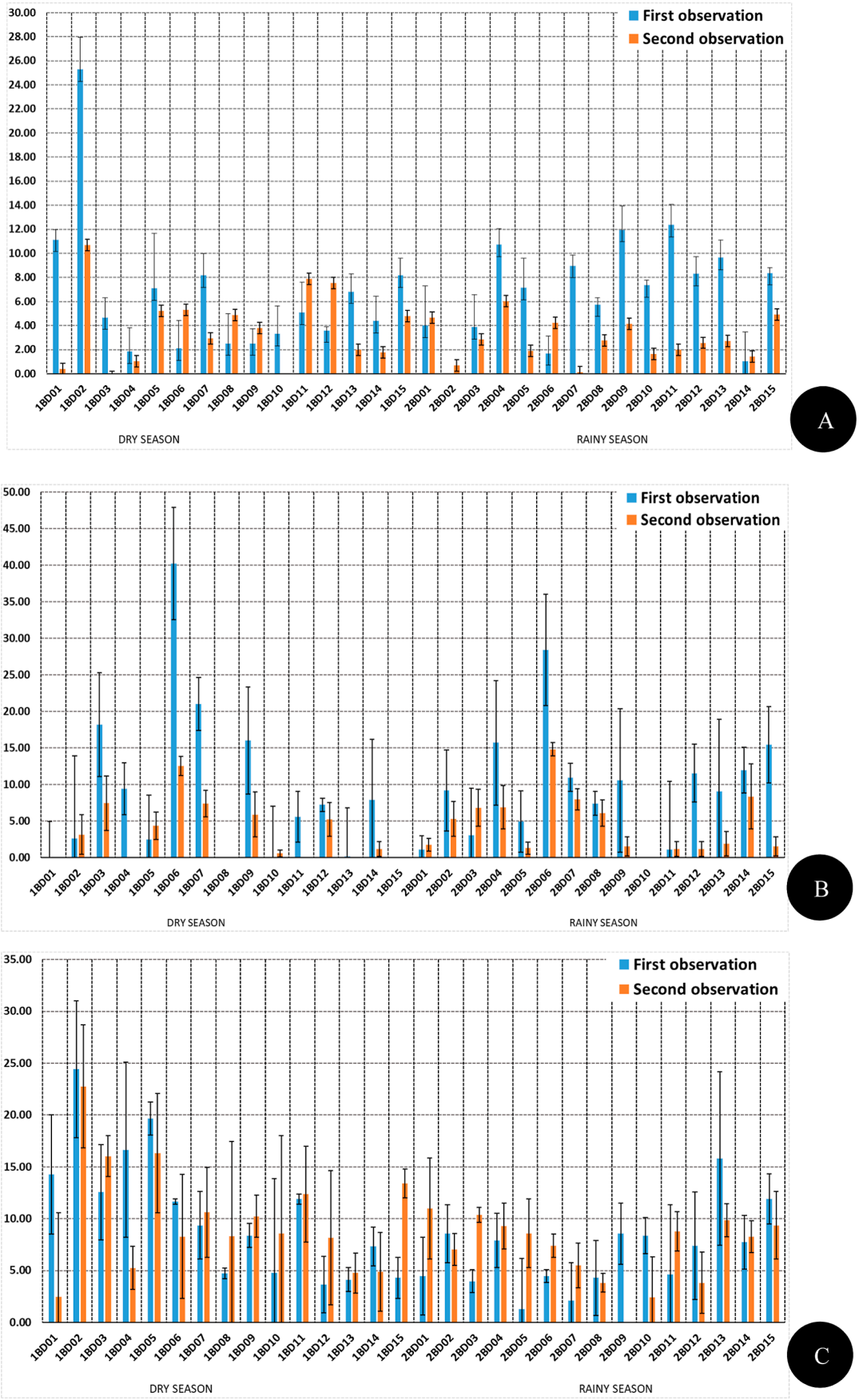

Figure 10. Percentage inhibition by essential oils of *B. dracunculifolia* collected in the dry and rainy seasons against (A) *A. nomius*, inoculum concentration 3.36×10^{-6} spores/mL; (B) *F. graminearum*, inoculum concentration 4.1×10^{6} spores/mL; and (C) *F. verticillioides*, inoculum concentration 11.6×10^{6} spores/mL.

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Author Contributions

P.H.F.: Conceptualization, methodology, formal analysis (statistical), data curation, investigation (SEM, antifungal, GC×GC), and writing—original draft. J.C.L.S.: Formal analysis (statistical), and writing—review and editing. R.F.: Data curation, formal analysis (GC×GC), and methodology (technical support). D.M.S.: Methodology (sample processing, laboratory support). M.N.F.C.: Methodology (laboratory support), and writing—review and editing. T.M.R.: Writing—review and editing, and formal analysis (SEM). A.R.M.: Writing—review and editing, data curation, and formal analysis (SEM). E.M.G.: Writing—review and editing, data curation, and formal analysis (antifungal). M.O.M.M.: Conceptualization, supervision, project administration, funding acquisition, and writing—review and editing. All authors have read and agreed to the published version of the manuscript.

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Notes

The authors declare no competing financial interest.

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