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Occurrence of Volatile Organic Compounds and Extrafloral Nectaries in Tropical Rainforest Species in Danum Valley Conservation Area, Sabah, Malaysia

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Abstract

Plants synthesize numerous classes of secondary metabolites that are crucial in plant defense. Two of the common but non-ubiquitous defenses are the emission of volatile organic compounds (VOCs) and production of extrafloral nectaries (EFN). This study investigates the occurrence of emission of VOC and production of EFN in forest species in Danum Valley Conservation Area, Sabah, Malaysia. Of the 165 species screened, 131 species were found to emit VOC while 41 species were EFN-bearing plants. There are 34 species that are both emitting VOC and producing EFN, while 97 species were found to be emitting VOC with no EFN observed. On the other hand, there are 7 species that were EFN bearing but non-VOC emitter, while 27 species were neither emitting VOC nor producing EFN. All 12 dipterocarp species were observed to emit VOC, of these 3 are non-EFN bearing. VOC emissions were further classified into isoprene (C5) and monoterpene (C10) compounds. There are 46 species that were detected to emit both isoprene and monoterpenes, while there are more exclusive monoterpene emitters (62 species) than isoprene-only emitters (23 species). This study showcased the ability of plants in producing a wide array of secondary metabolites as plant defense making them successfully adapt to the complexities of tropical rainforest ecosystem.

1. Introduction

Plants being immobile organisms are far from being defenseless. They have successfully adapted and evolved in hostile environments by developing mechanical, structural [1], [2] and chemical defense strategies [3]. Plants can synthesize numerous classes of secondary metabolites that accumulate during development and are thought to function as constitutive and/or facultative defense against herbivores and pathogens [4], [5] and in certain cases, protection against extreme environmental conditions [6]. In the current advancement of analytical laboratory techniques, many of these plant secondary metabolites originally produced for chemical defense become vital source of phytomedicines with numerous pharmaceutical and nutraceutical applications [7], [8], [9], [10].

Tropical rainforest is rich with biological diversity where mutualistic and antagonistic relationships between and among species are omnipresent. However, the non-ubiquitous occurrence of EFN and VOC vary and the specific selection by the plant of this role in plant defense strategies still puzzles scientists and researchers.

First observed by [11], [12] in tropical species, extrafloral nectaries are nectar-secreting glands that are not involved in pollination. EFN attracts insects like ants and wasps that their presence in nectary-bearing plants was observed to protect plants against herbivores thereby significantly increasing plant fitness [13], [14], [15], [16]. In effect, these plants have devised an 'attracting-the-enemy-of-my-enemy' mechanism underscoring the mutualistic association between nectary-bearing plants and ants [17]. In several *Macaranga* species, this kind of relationship reaches a certain high degree of sophistication in the so-called myrmecophytes (i.e. obligate ant-plant relationship) where plants offer shelter and food for their ant partners and return ants protect the plants from potential herbivory [17].

Another non-ubiquitous, more complex defense strategy observed in some species of plants is the production and emission of biogenic volatile organic compounds (VOC). Among the many biogenic VOC, volatile terpenoids produced by plants in relation to herbivore feeding include monoterpenes (C10), sesquiterpenes (C15), and homoterpenes (C11 or C16). These were first investigated by [18], [19], [20], among others. When volatilized, biogenic VOC can also act as signals for pollinators and conspecific herbivores [21], [22]. Moreover, the production of isoprene (C5) provides plants stability and protection against high temperature, increased solar radiation episodes, and potential ozone damage [23], [24], [25].

As production of these secondary metabolites are costly and entails partitioning of limited resources between growth and plant defense for the plant, it is hypothesized that plants may need to favor the production of one from the other [26]. To date, there is no strict categorization of what defense mechanism is favored by specific plant taxa, in particular tropical forest species. Hence, this paper investigates the production of two common, yet non-ubiquitous plant defense strategies in forest species – production of extrafloral nectaries (EFN) and emission biogenic volatile organic compounds (VOC) in the tropical rainforest of Danum Valley Conservation Area, Sabah, Malaysia. This will help ecologists, plant physiologists, and even plant pathologist in understanding the strategies of tropical forest species in terms of plant-herbivore interaction and plant defense in general, which may have application of nature-based solutions in the field of forestry (i.e. ecological succession, restoration, rehabilitation) as well as agroforestry and forest plantations.

2. Methodology

2.1 Study Site

The study was conducted in Danum Valley Conservation Area (DVCA, 4°58'N, 117°48'E), Sabah, Malaysia. DVCA comprises a large block (43.8 km2) of pristine lowland tropical rainforest estimated to be at least 130 million years old [27]. DVCA is one of the best representations of primary lowland dipterocarp forest boasting rich communities of flora and fauna with many species being endemic to the island of Borneo [28]. This ecosystem is also known for harboring the tallest tree in the tropics, the towering 100.8-meter *Shorea faguetiana*, locally known as Yellow Meranti [29]. Other dominant non-dipterocarp species recorded belong to the families Meliaceae, Anacardiaceae, Annocaceae, Leguminosae, Lauraceae, Euphorbiaceae, Myrtaceae, Tiliaceae, Sapindaceae, Datiscaceae, Fagaceae, and Burseraceae [30].

2.2 Screening of Species for BVOCs Content and Presence of EFN

Plants species were selected based on their dominance in Danum Valley Conservation Area. The identification of plant species was assisted by the Royal Society resident botanist/scientist, Mr. Bernadus Bala Ola (aka Mike). Classification and scientific names were verified using the World Online Flora (2023), as well as the Tree Flora of Sabah and Sarawak [31]. Collection of air samples and analysis were conducted following the methods described by [32] with modifications. Air samples were drawn from the modified leaf chamber of the Li-COR 6400 portable photosynthesis system (Li-Cor, Inc. Lincoln, Nebraska, USA) attached to a healthy, fully expanded leaf of the target plant species. Air sample from the leaf chamber was drawn using portable pump (SKC Pocket Pump Sampler, Pennsylvania, USA) for 20 minutes at a flow rate of 150 mL/min into the steel sampling tube (6.1 mm outside diameter, 90 mm length, Perkin Elmer, England). The inlet and outlet of the steel tubes were packed with Tenax TA (200 mg) and Carbotrap (100 mg), respectively. Both Carbotrap and Tenax TA have high affinity for compounds with a high range of boiling points from 60 to 300°C, hence are suitable for hydrocarbons and biogenic VOC from C_5 to C_{26} compounds [33]. However, for the purpose of this study, only isoprene and monoterpenes are getting attention. After collection, the steel tubes were stored in cold storage until analysis.

Blank samples were also taken to serve as control and subsequently used as correcting factor. While drawing air samples, assimilation (mol CO_2 m^{-2} s^{-1}) was recorded, along with other parameters such as humidity, temperature, CO_2 concentration, and photosynthetic photon flux density.

Plants sampled for BVOCs were visually observed for the presence/absence of extrafloral nectary glands, which are usually located at the base of the petiole/petiolule, stipules, or at the rachis as in case of plants with compound leaves. EFN glands can be present abaxially or adaxially. In all plants where glands were observed, none exhibited a fresh flow of nectar, therefore, no further analysis on the composition of nectar was conducted.

2.3 Analysis of Air Samples

Analysis of air samples followed the method described by [32] with minor modifications. Collected air samples were analyzed using gas chromatography with flame ionization detection (GC-FID) at Lancaster University, England, United Kingdom and at the Centre for Ecology and Hydrology, Edinburgh, Scotland, United Kingdom. Desorption and analysis of VOCs was carried out using Perkin Elmer ATD 400 thermal desorption unit connected via a transfer line heated to 200°C to a Hewlett Packard 5890A GC-FID with a 5890 mass-selective detector (GC-MSD).

Volatile organic compounds were desorbed at 280°C for 5 min at 25 ml min^{-1} onto a Tenax-TA cold trap maintained at -30°C. Secondary desorption was at 300°C for 6 min onto the GC column. Separation of the compounds was achieved using an Ultra-2 column (50 m x 0.2 mm x 0.11 mm ID, 5% phenylmethyl silica). The initial oven temperature of 35°C was maintained for 2 min, then increased at 4°C min-1 to 160°C followed by an increase of 45°C min^{-1} to 300°C which was maintained for 10 min. The carrier gas was helium at ~1 ml min^{-1}, the injector temperature was set at 250°C. For this system, the limit of detection for isoprene and monoterpenes was approximately 0.25 and 2 ng on column, respectively corresponding to 100 and 50 parts per trillion volume (pptv) of isoprene and monoterpenes in air for a 1 L sample. The level of analytical precision was around 6.5% for isoprene and 5% for monoterpenes.

Monoterpene quantification was by comparison with commercially available liquid standards (Aldrich, Fluka and Sigma) appropriately diluted and isoprene quantification by comparison with a 1 part per million volume (ppmv) in N_2 certified gas standard (Air products UK). Chemstation for Microsoft Windows was used to handle chromatographic data. Identification was achieved by comparison of retention times and mass spectra of authentic standards.

3. Result and Discussion

A total of 165 species, 134 genera in 64 families were screened for extrafloral nectary production and emission of volatile organic compounds. Table 1, Table 2, Fig. 1, and Fig. 2 summarize the number of species found to produce VOC and EFN, highlighting that there are more VOC-emitting plants (131 species) than EFN-bearing plants (41 species). There are 34 species that were observed to emit VOC and bear EFN gland, while 27 species neither emit VOC nor exhibit EFN gland. A total of 97 species were known to emit VOC but has no observed EFN gland while 7 species exhibited EFN gland but had no VOC emission detected.

Table 1 *Occurrence of isoprene and monoterpenes on volatile organic compound emitting plants in Danum Valley Conservation Area, Sabah, Malaysia*

Taxon / Habit	VOC-Emitting	EFN-bearing	Total	
			No.	%
Species [165]	Yes	Yes	34	20.60
	Yes	No	97	58.80
	No	Yes	7	4.24
	No	No	27	16.36
			165	**100.00**
Tree [134 sp]	Yes	Yes	25	18.66
	Yes	No	80	59.70
	No	Yes	5	3.73
	No	No	24	17.91
			134	**100.00**
Shrub [15 sp]	Yes	Yes	2	13.33
	Yes	No	9	60.00
	No	Yes	1	6.67
	No	No	3	20.00
			15	**100.00**
Herb [9 sp]	Yes	Yes	5	55.56
	Yes	No	3	33.33

	No	Yes	1	11.11
	No	No	0	0.00
			9	**100.00**
Bamboo [2 sp]	Yes	Yes	2	100.00
	Yes	No	0	0.00
	No	Yes	0	0.00
	No	No	0	0.00
			2	**100.00**
Palm [2 sp]	Yes	Yes	0	0.00
	Yes	No	2	100.00
	No	Yes	0	0.00
	No	No	0	0.00
			2	**100.00**
Climbers [3 sp]	Yes	Yes	0	0.00
	Yes	No	3	100.00
	No	Yes	0	0.00
	No	No	0	0.00
			3	**100.00**

Table 2 *List of species screened for VOC emission and EFN glands in Danum Valley Conservation Area, Sabah, Malaysia*

	FAMILY	SPECIES	HABIT	VOC EMITTER	EFN BEARING
1.	Alangiaceae	*Alangium javanicum* (Blume) Wangerin	Tree	y	n
2.	Anacardiaceae	*Buchanania insignis* Blume	Tree	y	n
3.	Anacardiaceae	*Mangifera indica* L.	Tree	y	n
4.	Anacardiaceae	*Tapirira guianensis* Aubl.	Tree	n	n
5.	Annonaceae	*Annona senegalensis* Pers.	Tree	n	y
6.	Annonaceae	*Anonidium mannii* (Oliv.) Engl. & Diels	Tree	n	n
7.	Annonaceae	*Greenwayodendron suaveolens* (Engl. & Diels) Verdc.	Tree	n	n
8.	Annonaceae	*Guatteria dumetorum* R.E. Fr.	Tree	n	n
9.	Annonaceae	*Maasia sumatrana* (Miq.) Mols, Kessler & Rogstad	Tree	y	n
10.	Annonaceae	*Monodora myristica* (Gaertn.) Dunal	Tree	n	n
11.	Annonaceae	*Polyalthia insignis* (Hook.f.) Airy Shaw	Tree	y	n
12.	Annonaceae	*Polyalthia* sp. *1*	Tree	y	n
13.	Annonaceae	*Polyalthia* sp. *2*	Tree	y	n
14.	Annonaceae	*Xylopia hypolampra* Mildbr.	Tree	y	n
15.	Apocynaceae	Apocynaceae sp.	Tree	y	n
16.	Apocynaceae	*Tabernaemontana* sp. 1	Shrub	y	y
17.	Arecaceae	*Arenga undulatifolia* Becc.	Palm	y	n
18.	Arecaceae	*Elaeis guineensis* Jacq.	Palm	y	n
19.	Burseraceae	Burseraceae sp.	Tree	y	n
20.	Burseraceae	*Canarium odontophyllum* Miq.	Tree	y	n
21.	Burseraceae	*Dacryodes rugosa* (Blume) H.J.Lam	Tree	y	n
22.	Burseraceae	*Protium pittieri* (Rose) Engl.	Tree	y	n
23.	Celastraceae	Celastraceae sp.	Herb	y	n

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24.	Celastraceae	*Cophopetaum sp.*	Vine	y	n
25.	Chrysobalanaceae	*Parinari oblongifolia* (Hook.f)	Tree	y	n
26.	Clusiaceae	*Calophyllum obliquinervium* Merr.	Tree	y	n
27.	Clusiaceae	*Calophyllum* sp.	Tree	y	n
28.	Clusiaceae	Clusiaceae sp.	Tree	y	n
29.	Clusiaceae	*Garcinia mangostana* L.	Tree	y	n
30.	Combretaceae	Combretaceae sp.	Tree	y	n
31.	Cornaceae	Cornaceae sp.	Shrub	y	n
32.	Datiscaceae	*Octomeles sumatrana* Miq.	Tree	y	y
33.	Dilleniaceae	Dilleniaceae sp. 1	Tree	y	n
34.	Dilleniaceae	Dilleniaceae sp. 2	Tree	n	n
35.	Dipterocarpaceae	*Dipterocarpus caudiferus* Merr	Tree	y	n
36.	Dipterocarpaceae	*Dryobalanops lanceolata* Burck.	Tree	y	n
37.	Dipterocarpaceae	*Hopea nervosa* King	Tree	y	n
38.	Dipterocarpaceae	*Parashorea tomentella* (Sym.) Meijer.	Tree	y	y
39.	Dipterocarpaceae	*Shorea agami* P.Ashton	Tree	y	y
40.	Dipterocarpaceae	*Shorea fallax* Meijer	Tree	y	y
41.	Dipterocarpaceae	*Shorea leprosula* Miq.	Tree	y	y
42.	Dipterocarpaceae	*Shorea oleosa* Meijer	Tree	y	y
43.	Dipterocarpaceae	*Shorea parvifolia* Dyer	Tree	y	y
44.	Dipterocarpaceae	*Shorea symingtonii* G.H.S. Wood	Tree	y	y
45.	Dipterocarpaceae	*Vatica sarawakensis* Heim.	Tree	y	y
46.	Ebenaceae	*Diospyros* sp.	Tree	y	y
47.	Ebenaceae	*Diospyros squamifolia* Kosterm.	Tree	y	y
48.	Elaeocarpaceae	Elaeocarpaceae sp.	Tree	y	n
49.	Euphorbiaceae	*Alchornea cordifolia* (Schumach. & Thonn.) Müll. Arg.	Tree	y	y
50.	Euphorbiaceae	*Alchornea costaricensis* Pax & K.Hoffm.	Tree	n	y
51.	Euphorbiaceae	*Baccaurea angulata* Merr	Tree	y	n
52.	Euphorbiaceae	*Baccaurea stipulata* J.J. Sm.	Tree	y	n
53.	Euphorbiaceae	*Hevea brasiliensis* Müll. Arg.	Tree	y	n
54.	Euphorbiaceae	*Koilodepas longifolium* Hook.f.	Tree	y	y
55.	Euphorbiaceae	*Macaranga hypoleuca* (Rchb.f. & Zoll) Müll.Arg.	Tree	y	y
56.	Euphorbiaceae	*Macaranga pearsonii* Merr.	Tree	y	y
57.	Euphorbiaceae	*Macaranga triloba* (Thunb.) Müll. Arg.	Tree	y	y
58.	Euphorbiaceae	*Mallotus korthalsii* Müll.Arg	Tree	y	y
59.	Euphorbiaceae	*Mallotus paniculatus* (Lam) Müll. Arg.	Tree	y	y
60.	Euphorbiaceae	*Mallotus* sp. *2*	Tree	y	y
61.	Euphorbiaceae	*Mallotus* sp.*1*	Tree	y	y
62.	Euphorbiaceae	*Mallotus wrayi* King ex Hook.f.	Tree	y	y
63.	Euphorbiaceae	*Manihot ultissima* Pohl	Shrub	n	y
64.	Euphorbiaceae	*Manniophyton fulvum* Müll. Arg.	Shrub	n	n

65.	Euphorbiaceae	*Pera arborea* Baill.	Tree	n	n
66.	Fagaceae	*Lithocarpus* sp.	Tree	y	n
67.	Flacourtiaceae	*Caloncoba welwitschii* (Oliv.) Gilg	Tree	n	n
68.	Flacourtiaceae	*Hydnocarpus borneensis* Sleumer	Tree	y	y
69.	Flacourtiaceae	*Ryparosa hulettii* King	Tree	y	y
70.	Icacinaceae	*Stemonurus scorpioides* Becc.	Tree	y	n
71.	Lauraceae	*Beilschmiedia micrantha* Merr	Tree	y	n
72.	Lauraceae	*Litsea firma* (Blume) Hook.f.	Tree	y	n
73.	Lauraceae	*Nectandra purpurea* (Ruiz &Pav.) Mez.	Tree	n	n
74.	Lauraceae	*Persea americana* Mill.	Tree	n	n
75.	Lecythidaceae	*Barringtonia lanceolata* (Ridl.) Payens	Tree	y	n
76.	Lecythidaceae	*Barringtonia sarcostachys* (Blume) Miq.	Tree	y	n
77.	Leeaceae	Leeaceae sp.	Shrub	y	n
78.	Leguminosae	*Acacia nigrescens* Oliv.	Tree	y	y
79.	Leguminosae	*Acacia tortilis* (Forssk.) Hayne	Tree	y	y
80.	Leguminosae	*Fordia splendidissima* (Miq.) Buijsen	Tree	y	n
81.	Leguminosae	*Koompassia excelsa* (Becc.) Taub.	Tree	y	n
82.	Leguminosae	*Sindora irpicina d*e Wit	Tree	y	n
83.	Loganiaceae	*Fagraea cuspidata* Blume	Tree	y	n
84.	Loganiaceae	Loganiaceae sp.	Tree	y	n
85.	Magnoliaceae	*Magnolia liliifera* (L.) Baill.	Tree	y	n
86.	Malvaceae	*Ceiba pentandra* (L.) Gaertn.	Tree	n	y
87.	Malvaceae	*Durio* sp.	Tree	y	n
88.	Melastomataceae	*Miconia impetiolaris* (Sw.) D. Don ex DC	Shrub	n	n
89.	Melastomataceae	*Miconia* sp.	Shrub	n	n
90.	Melastomataceae	*Pternandra caerulescens* Jack	Shrub	y	n
91.	Melastomataceae	*Pternandra* sp.	Shrub	y	n
92.	Meliaceae	*Carapa guianensis* Aubl.	Tree	n	n
93.	Meliaceae	*Chisocheton* sp.	Tree	y	n
94.	Meliaceae	*Entandrophragma utile* (Dawe & Sprague) Sprague	Tree	n	n
95.	Meliaceae	*Guarea* sp.	Tree	n	n
96.	Meliaceae	*Trichilia gilgiana* Harms	Tree	n	y
97.	Monimiaceae	Monimiaceae sp.	Herb	y	n
98.	Moraceae	*Brosimum utile* (Kunth) Oken	Tree	y	n
99.	Moraceae	*Ficus fistulosa* Reinw. ex. Bl.	Tree	y	n
100.	Moraceae	*Ficus glumosa* Delile	Tree	y	n
101.	Moraceae	*Ficus nymphaefolia* Mill.	Tree	y	n
102.	Moraceae	*Perebea xanthochyma* H. Karst.	Tree	y	n
103.	Moraceae	*Poulsenia armata* (Miq.) Standl.	Tree	n	n
104.	Moraceae	*Trilepisium madagascariense* DC.	Tree	y	n
105.	Musaceae	*Musa beccarii* N.W. Simmonds	Herb	y	n

106.	Myristicaceae	*Coelocaryon botryoides* Vermoesen	Tree	n	n
107.	Myristicaceae	*Knema* sp.1	Tree	y	n
108.	Myristicaceae	*Knema* sp.2	Tree	y	n
109.	Myristicaceae	*Virola sebifera* Aubl.	Tree	y	n
110.	Myristicaceae	*Virola* sp.	Tree	y	n
111.	Myrsinaceae	*Ardisia* sp.	Shrub	y	y
112.	Myrtaceae	*Syzygium grande* (Wight) Walp.	Tree	y	n
113.	Myrtaceae	*Syzygium guineense* (Willd.) DC	Tree	y	n
114.	Myrtaceae	*Syzygium kunstleri* (King) Bahadur & R.C. Gaur	Tree	y	n
115.	Myrtaceae	*Syzygium paniculatum* Gaertn.	Tree	y	n
116.	Myrtaceae	*Syzygium* sp.	Tree	y	n
117.	Ochnaceae	Ochnaceae sp.	Tree	y	n
118.	Olacaceae	Olacaceae sp.	Tree	y	n
119.	Oleaceae	Oleaceae sp.	Tree	y	n
120.	Poaceae	*Bambusa vulgaris* Schrad.	Bamboo	y	y
121.	Poaceae	*Schizostachyum brachycladium* (Kurz) Kurz	Bamboo	y	y
122.	Polygalaceae	*Xanthophyllum* sp.	Herb	y	y
123.	Proteaceae	Proteaceae sp.	Vine	y	n
124.	Rhamnaceae	*Ziziphus angustifolius* King & Gamble	Vine	y	n
125.	Rhizophoraceae	Rhiziphoraceae sp.	Tree	y	n
126.	Rosaceae	Rosaceae sp.	Tree	y	n
127.	Rubiaceae	*Coffea* sp.	Tree	n	n
128.	Rubiaceae	*Corynanthe mayumbensis* (R.D. Good) N. Halle	Tree	n	n
129.	Rubiaceae	*Praravinia suberosa* (Merr.) Bremek	Tree	y	n
130.	Rubiaceae	*Tocoyena pittieri* (Standl.) Standl.	Tree	n	y
131.	Rubiaceae	*Warszewiczia coccinea* (Vahl) Klotzsch	Tree	n	n
132.	Rutaceae	*Zanthoxylum gilletii* (De Wild.) P.G. Waterman	Tree	n	n
133.	Sabiaceae	Sabiaceae sp.	Shrub	y	n
134.	Santalaceae	Santalaceae sp.	Shrub	y	n
135.	Sapindaceae	*Allophylus africanus* P. Beauv.	Tree	n	n
136.	Sapindaceae	*Dimocarpus longan* Lour.	Tree	y	n
137.	Sapindaceae	*Nephelium mutabile* Blume	Tree	y	n
138.	Sapindaceae	*Nephelium ramboutan-ake* (Labill.) Leenh.	Tree	n	n
139.	Sapindaceae	*Pancovia laurentii* (De Wild.) Gilg. Ex De Wild.	Tree	y	n
140.	Sapindaceae	*Paranephelium xestophyllum* Miq.	Tree	y	n
141.	Sapotaceae	*Manilkara bidentata* (A.DC.) A. Chev.	Tree	n	n
142.	Sapotaceae	*Payena acuminata* (Blume) Pierre	Tree	y	n
143.	Saurauiaceae	Saurauiaceae sp.	Shrub	y	n
144.	Saxifragaceae	Saxifragaceae sp.	Shrub	y	n
145.	Simaroubaceae	*Simarouba amara* Aubl.	Tree	n	n
146.	Simaroubaceae	Simaroubaceae sp.	Tree	y	n

147.	Sonneratiaceae	*Duabanga moluccana* Blume	Tree	y	n
148.	Sterculiaceae	*Pterospermum* sp.	Tree	y	n
149.	Styracaceae	Styracaceae sp.	Shrub	y	n
150.	Symplocaceae	Symplocaceae sp.	Tree	y	n
151.	Symplocaceae	*Symplocos fasciculata* Roxb. ex A.DC.	Tree	y	n
152.	Theaceae	Theaceae sp.	Tree	y	n
153.	Thymelaeaceae	*Aquilaria malaccensis* Lam.	Tree	y	n
154.	Tiliaceae	*Apeiba membranacea* Spruce ex Benth.	Tree	n	n
155.	Tiliaceae	*Microcos crassifolia* Burret	Tree	y	n
156.	Tiliaceae	*Pentace laxiflora* Merr.	Tree	y	n
157.	Tiliaceae	*Pentace* sp.	Tree	y	n
158.	Trigoniaceae	Trigoniaceae sp.	Tree	y	n
159.	Ulmaceae	Ulmaceae sp.	Tree	y	n
160.	Verbenaceae	*Callicarpa pentandra* Roxb.	Tree	y	n
161.	Zingiberaceae	*Etlingera brachychila* (Ridl.) R.M.Sm	Herb	y	y
162.	Zingiberaceae	*Etlingera brevilabrum* (Valeton) R.M.Sm	Herb	y	y
163.	Zingiberaceae	*Etlingera coccinea* (Blume) S.Sakai & Nagam	Herb	y	y
164.	Zingiberaceae	*Etlingera elatior* (Jack) R.M. Smith	Herb	n	y
165.	Zingiberaceae	*Etlingera littoralis* (J. Koenig) Giseke	Herb	y	y

Notes: y = YES; n = NO
Classification and species names were cross-checked with the World Flora Online accessed on September 2023 <http://www.worldfloraonline.org/>, previously known as The Plant List.

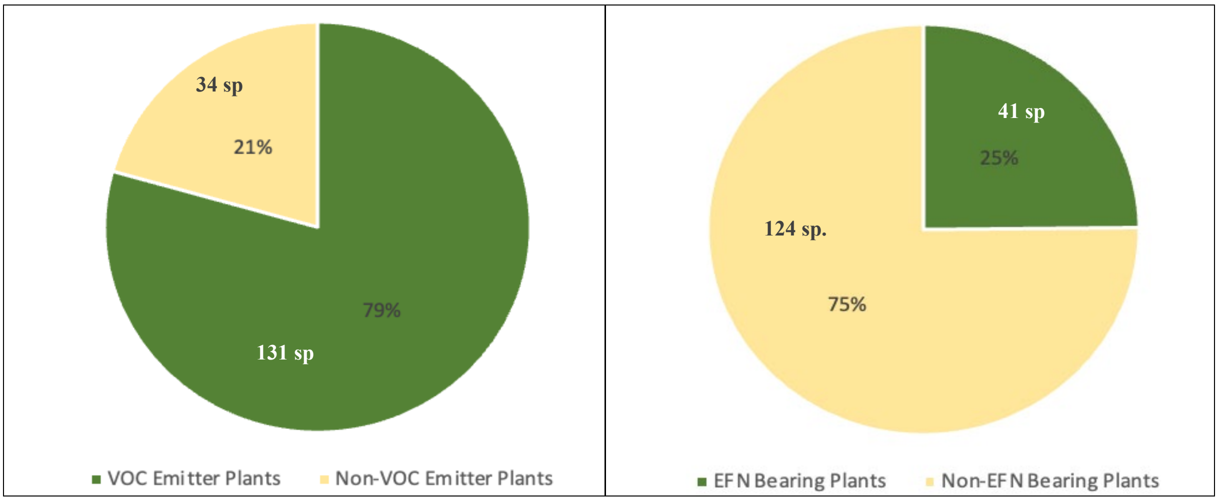

Fig. 1 *Number of plants detected to emit volatile organic compounds (VOC) and observed to bear extrafloral nectaries (EFN) in Danum Valley Conservation Area, Sabah, Malaysia*

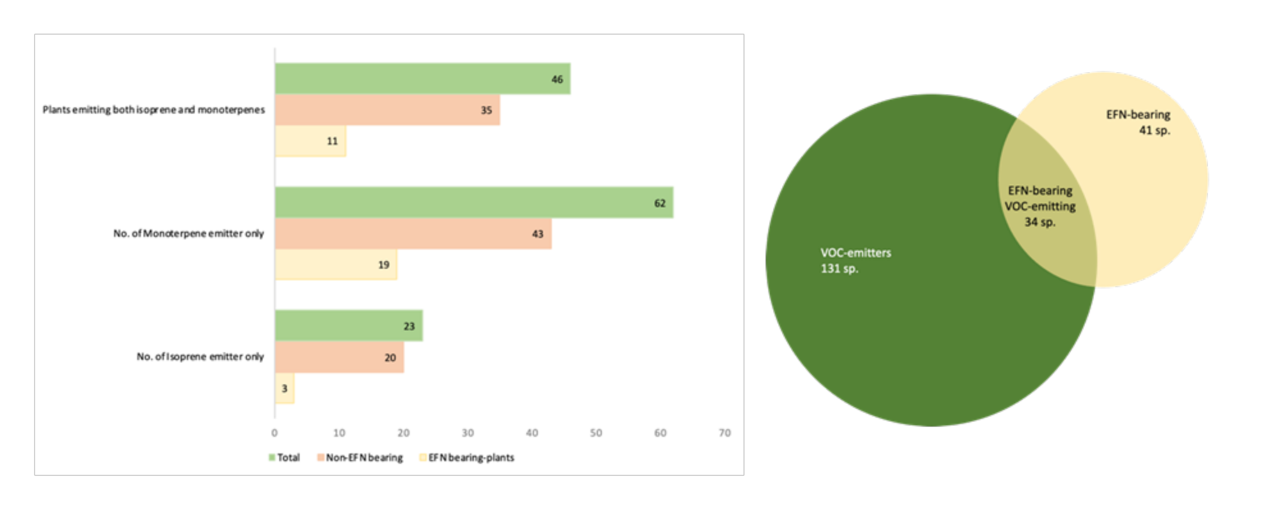

Fig. 2 *Breakdown of VOC-emitting and EFN-bearing plants in Danum Valley Conservation Area, Sabah, Malaysia*

Of the 131 VOC-emitting species, 23 species were found to emit isoprene only, 62 species to be exclusive monoterpene-emitters, while 46 species were found to emit both isoprene and monoterpenes (Fig. 1, Fig. 2, Table 3). In contrast, only 41 species (24.8%) of the 165 species screened are EFN-bearing plants (Fig. 2). This trend supports the affinity of plants to spend their limited resources to the production of secondary metabolites that provide plants with wide array of benefits [34]. Volatile organic compounds, whether induced or constitutively present were proven to mediate interactions between plants and the environment by attracting pollinators, repelling herbivores, protecting against microorganism, and protecting plants against stress, oxidation, and high temperatures [22,] [23], [24], and [35].

Table 3 *Emission of isoprene and monoterpenes in plants in Danum Valley, Sabah, Malaysia*

VOC emitting plants	No. of Isoprene emitter only	No. of Monoterpene emitter only	Plants emitting both isoprene and monoterpenes
EFN bearing-plants	3	19	11
Non-EFN bearing	20	43	35
Total	23	62	46

The number of plants (85 species) exclusively emitting either isoprene (C5 compounds) and monoterpenes (C10 compounds) and not found to bear or produce extrafloral nectaries (Table 1) is two-fold more than plants producing both EFN and VOCs (34 species). This result is an indicative of plant's flexibility in allocating resources for plant defense responses [26], [36]. Some species are allocating a wide array of defenses including production of chemical barriers in the form of toxic secondary metabolites and defense proteins that can repel, suppress oviposition and feeding, and hinder the digestion of herbivores, while other species are favoring indirect defense mechanism such as the attraction of parasitoids and predators through structures that offer food and protection (i.e., EFNs and domatia) [34]. The ability of 8 out of 11 Dipterocarp species to exhibit production of VOCs and EFN (Table 1), while the three other species were found to emit VOCs with undetected EFN supports the optimal defense hypothesis that is said to maximize fitness [37], a trait that may have significantly contributed to the species' ecological success [38]. Species belonging to the Euphorbiaceae exhibit a relatively unpredictable pattern than that of Dipterocarpaceae. Ten out of 17 species of Euphorbs were detected to have both EFN and VOC production; two species were not detected to have either VOC or EFN; three species are exclusive VOC emitter, while two are exclusive EFN producer.

Volatile organic compounds play various ecological roles. Isoprene (5-carbon atoms) is known to provide thermal protection in plants by increasing the plant's tolerance of photosynthesis to high temperature by stabilizing the thylakoid membrane [39] or by quenching reactive oxygen species [23]. On the other hand, monoterpenes (10-carbon atoms or with 2-isoprene units) have at least 400 structures and the most diverse form of biogenic volatile organic compounds [40]. Many of these monoterpenes and monoterpenoids constitute approximately 90% of essential oils demonstrating a wide range of biological activities, which proved to have pharmacological (i.e., antibacterial, anti-inflammatory, hypotensive, antipruritic) and nutraceutical applications (flavors and fragrances) [40]. In terms of plant defense, monoterpenes are known to benefit plants by attracting or repelling natural enemies, suppress feeding and oviposition, toxic and antimicrobial properties, and mediate plant-to-plant communication or even between parts of the same plant, thereby increasing overall plant fitness [40]. These properties make isoprene and monoterpenes ubiquitous to many C3 plants including temperate and tropical species [35], [41]. In this study, 69 out of 131 species are isoprene emitters (52.67%) relatively fewer than 108 monoterpene emitters (82.44%) (Table 3.). This result supports the studies by [41] stating that biogenic VOC emission from tropical vegetations is estimated to be responsible for >70% of global biogenic VOC emission.

The non-emitter plants in this study do not necessarily categorize as non-VOC producing. In some species, production and emission of some VOCs are known to be induced or are affected by the growth environment and/or developmental stage of the plant [41], [42], [43]. Moreover, diurnal variations are also common occurrence observed in some species when emissions were highest in the morning as the air temperature and light intensity increases [41]. Isoprene emissions were confined to daytime only [44], [45], [46] while monoterpenes were detected at night [45].

In terms of EFN production, though glands were obvious and visible to 41 species, no fresh liquid was evident during the entire study, however the presence of ants were observed in all plants. Mutualist association between EFN producing plant and insects have been well-established [47]. Unlike VOCs that serve direct and indirect defense strategy, EFN is produced by plants in a form of exclusive reward to organisms, usually ants for protecting the plant from herbivores [48]. The extrafloral nectar produced by plants is a liquid secreted on different aboveground parts of the plant usually comprising an aqueous solution with high levels of mono- and disaccharides and lower levels of amino acids, lipids, and enzyme which together fulfill nutritive functions for diverse nectar consumer [49], [50]. The role of EFN in tritrophic interaction needs more investigation, especially in a diverse and complex tropical ecosystem like Danum Valley Conservation Area (DVCA). Several studies have suggested that plants have the ability to recruit natural enemies of herbivores by having induced EFN production [51]. The two most dominant families in DVCA have similar patterns of species bearing both EFN and VOCs as majority of the species observed in each family were capable to produce both VOC and EFN gland (Fig. 2, Table 3). This is one of the features that might support the ecological success of these taxa in a complex, highly competitive tropical rainforest [34]. Ten species of Euphorbiaceae were observed to have both EFN glands and VOCs, whereas 2 species are non-VOC emitter but were observed to bear EFN glands; neither EFN nor VOC was observed in five species.

4. Conclusion and Recommendations

Plants are capable of manufacturing various defense strategies that protect itself from threats like herbivory and hostile environment. Two of these defense mechanisms observed in plants from Danum Valley Conservation Area are the production of extrafloral nectaries (EFN) and volatile organic compounds (VOC). Isoprene and monoterpenes were the class of volatile organic compounds considered in this study. It is now understood that plants are capable of producing a wide array of plant secondary metabolites that are beneficial to plant protection, hence it is recommended that further studies should be considered in determining other volatile organic compounds plants like sesquiterpenes (C15 compounds or 3-isoprene units) and other higher carbon-molecules plants are capable of producing to better understand how plants allocate limited resources as far as plant protection is concerned. Moreover, a long-term, more in-depth observation of plants bearing EFN glands is recommended, and proper timing of observation is crucial as the aqueous nectar produced by the plant can easily be devoured by associated ants or even evaporate. Identification of species of ants and other species involved in tritrophic interactions is crucial in better understanding of plant-insect interaction in tropical ecosystems.

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Conflict of Interest

Authors declare that there is no conflict of interests regarding the publication of the paper.

Author Contribution

The authors confirm contribution to the paper as follows: ***study conception and design:*** *,Alona C. Linatoc, Susan M. Owen, Mohd Noh Dalimin;* ***data collection:*** *Alona C. Linatoc;* ***analysis and interpretation of results:*** *Alona C.*

Linatoc; Susan M. Owen, Mohd Noh Dalimin ***draft manuscript preparation:*** *Alona C. Linatoc, Susan M. Owen, Mohd Noh Dalimin. All authors reviewed the results and approved the final version of the manuscript.*

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