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Short Communications

Edaphic Characteristics of *Rafflesia* Habitats in Indonesia: Implications for Conservation and Propagation

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ABSTRACT
Rafflesia, a holoparasitic and endophytic plant, depends on its host, *Tetrastigma* spp., for survival, thus highlighting the critical interdependence between these species. Given the endangered status of *Rafflesia* due to anthropogenic pressures and narrow distribution, comprehensive conservation efforts are crucial. Ecological data on edaphic conditions, particularly the presence of the host, are important for effective conservation strategies. This study assessed soil properties across *Rafflesia* habitats on Sumatera, Borneo, and Java islands, revealing similarities in pH, carbon, nitrogen, cation exchange capacity (CEC), while the soil texture varied. These findings contribute valuable insights for informed conservation initiatives, both in-situ and ex-situ.

Rafflesia is a unique and endemic plant species of Indonesia. It has single biggest flower among flowering plants species in the world. Despite being large in size, *Rafflesia* is actually a holoparasitic and endophytic plant without vegetative organs such as stems, leaves roots or any photosyntentic systems (Molina et al. 2014; Mursidawati et al. 2020). Hence its life cycle, survival and establishment depend on its host, which is known as *Tetrastigma* spp. (Vitaceae). Thus, the distribution of the *Rafflesia* spp. in the forest are entirely dependent on the existence of its host species and its supporting plants. Because these plants are influence each other, all plant forms (lifeforms) and their habitat must be equally conserved (Adnan & Hadisusanto 2023).

In Indonesia, *Rafflesia* can be found on three of the largest islands: Sumatra, Borneo, and Java, as well as on some smaller surrounding islands like Nusa Kambangan (van Steenis et al. 1954) and Mursala Island (Mahyuni et al. 2016). All the *Rafflesia* species in Indonesia are considered endangered due to their unique biological and ecological characteristics, as well as the impact of human activities (Hidayati & Walck 2016; Lestari & Susatya 2022). Therefore, ex-situ conservation of *Rafflesia* is strongly recommended as a solution, especially because it can be challenging to access *Rafflesia* in its natural habitat for research purposes (Lestari & Susatya 2022). Wicaksono et al. (2016) also proposed the artificial or human intervention cultivation of *Rafflesia* spp.

To propagate or conserve *Rafflesia* in ex-situ area, ecological data which provide conditions required for its growth are needed, especially the edaphic conditions where the host exists (Hayati et al. 2021). In previous study, Wahab et al. (2021) mentioned that the presence of *Tetrastigma* sp. in the different *Rafflesia* habitats has its own relationship with the soil and is not influenced by one factor. Therefore, a more comprehensive study related to the edaphic habitat of various *Rafflesia* species must be carried out.

In Indonesia, studies related to the edaphic habitat of *Rafflesia* are still limited and mostly focused on aspects like soil pH. For example, Ramadhani et al. (2017) found that the area where *Rafflesia arnoldi* is found are usually have temperatures between 25 and 29 degrees Celsius, humidity levels of 95%, and an acidic pH of 5.5, with the soil consists of sandy clay soil with varying percentages of sand, silt, and clay. *R. patma* in Bojonglarang Jayanti Nature Reserve grows in more acidic soil with a pH of 5.5 and pH KCl at 4.8, with soil classified as sandy clay soil with different percentages of sand, silt, and clay, with the organic nutrient content is higher compared to the *Tetrastigma* plot, while the inorganic nutrient content or mineral soil elements in the *Rafflesia* plot are lower (Ali et al. 2015). *R. patma* in Leweung Sancang live in regosol soil, sandy loam, with loose and well-drained soil, acidic to neutral soil pH, very high organic C and Ca content, total N, Mg, high CEC, very low available P, and moderate K and Na (Priatna et al. 1989). *R. patma* in Ciletuh also grows on acidic soils with pH ranging from 5.8 to 7 (Triana et al. 2017). Laksana et al. (2018) found that the types of soil in the permanent plots of *R. zollingeriana* in Krecek are latosol soil, generally dark reddish in colour, and with a clay texture, with soil pH considered neutral, as it is close to 7. Rambey et al. (2023) observed that the habitat of *R. meijeeri* in Batang Gadis National Park Pagar Gunung indicating a neutral soil pH as it is close to 7 overalls (6.8 – 6.9). But there are also studies that do not address soil acidity. In addition, Renjana et al. (2022) mentioned that soil organic carbon was the most important variable affecting the occurrence of the host plants of *R. arnoldii*.

However, these studies only provide partial information, and more comprehensive research is needed to fully understand the specific edaphic conditions preferred by different *Rafflesia* species. Therefore, this study aims to assess the physical and chemical properties of soil at three distinct locations where three different *Rafflesia* species are found on different islands in Indonesia: Java, Sumatera, and Borneo. By gaining deeper insights into the physical and chemical properties of the soil in the natural habitat of these *Rafflesia* species, we can identify the factors that contribute to their growth and survival. The information of edaphic characteristics also plays a crucial role in determining the suitable locations for these species to thrive and reproduce, as well as their susceptibility to environmental changes and disturbances. This knowledge can be used to develop effective conservation strategies, such as habitat restoration or management, to support the survival of these unique species in their natural environments.

The study was conducted in three different islands of Indonesia where four populations of *Rafflesia* spp. were identified (Table 1). These study locations comprise Tempursari-Lumajang in East Java, Palak Siring Kemumu in Bengkulu, Sumatera island and Kayan Mentarang National Park in North Kalimantan, which is located in the island of Borneo (Figure 1).

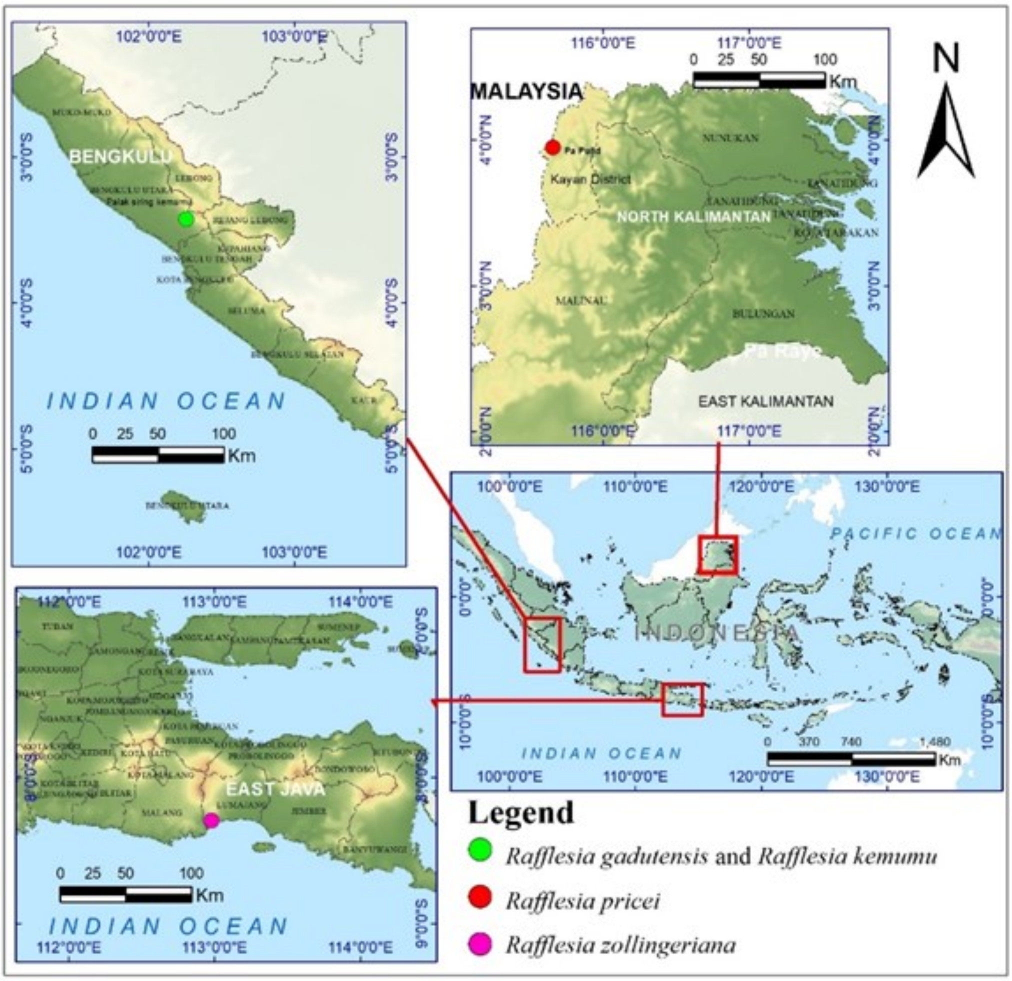

Figure 1. Map of Three *Rafflesia* Habitats in Three Island of Indonesia.

The first sample was collected from Argosari, a protected forest of Perhutani, that adjacent to human settlement in Tempursari, Lumajang, East Java, located at 450 m above sea level (asl) (Table 1). The new record for *R. zollingeriana* in East Java was detected from this area (Lestari & Susatya 2022).

Palak Siring Kemumu, Bengkulu Utara was habitat for two species *Rafflesia*: *R. gadutensis* and *R. kemumu* (Susatya et al. 2017). This area is lowland protected forest that also functioning as ecotourism object, located at 290 m a.s.l in Arga Makmur, Arma Jaya, Bengkulu Utara (Figure 2).

Kayan Mentarang National Park is lies between Malinau and Nunukan Regency, North Kalimantan, Indonesia. This national park is reported as habitat for *R pricei* (Jayasilan et al. 2004; Lestari et al. 2020). The soil sample was collected from *R. pricei* habitat in hillside of montane forest at 60^0 slopes, at 1277 m a.s.l. in Pa' Pulid forest near to Pa' Api, Nunukan, Kalimantan Utara.

Table 1. The study sites of four species of *Rafflesia*.

Location	Status	*Rafflesia* Species	Host Species
Tempursari, Lumajang	Conservation area	*R. zollingeriana*	*T. rafflesiae*
Palak Siring Kemumu	Conservation area for Ecotourism	*R. gadutensis*, *R. kemumu*	*T. leucostaphylum* and *T. pedunculare*
Kayan Mentarang National Park	National park	*R. pricei*	*Tetrastigma*. sp.

Figure 2. (A) *R. kemumu* blooms in Palak Siring, North Bengkulu (Picture by Bara); (B) Survey of *R. gadutensis* habitat; (C) First day blooming of *R. zollingeriana* in Lumajang (Picture by DL); (D) Survey of *R. zollingeriana* habitat. (E) Post blooming of *R. pricei* in Kayan Mentarang National Park (Picture by DL); (F) Survey of *R. pricei* habitat.

Soil sampling was carried out based purposive random sampling method in three different sites. Soil samples were collected from 0-20cm depth. After taken from the plots, the soil sample then air dried and analysed in Indonesian Coffee and Cocoa Research Institute (ICCRI) laboratory. The parameters soil were soil chemical and physical property. The chemical properties consist of soil pH, organic carbon (%), total nitrogen content (%), Phosphorus (ppm), K, Ca, Na, Mg (me $100g^{-1}$), Electron Exchange Capacity (CEC) (me $100g^{-1}$) and Base Saturation (%). Additionally, the observation of soil physical property focused on the soil texture.

Soil pH determine in water (1:2.5) using pH meter, organic carbon used wet oxidation method by Walkey and Black. Phosphorus extraction by Bray and Olsen method and K, Ca, Mg, Na, CEC and Base saturation through leaching with NH_4SO_4. Soil parameters ranking based on Indonesian Soil Research Institute (Balittanah 2009). The determination of soil texture was using the soil texture triangle (Figure 3). The percentage of each particles obtained then applied to the soil texture triangles and cross point between the percentage of particles to determine the texture class.

Based on our results, there were variations in soil texture and chemical properties among the three habitats of *Rafflesia*. The pH level in all three locations was found to be slightly acidic, ranging from 5.8 to 6. The Palak Siring Kemumu site had higher levels of total carbon and nitrogen content, with measurements of 7.67% and 0.69%, respectively, in comparison to the Tempursari-Lumajang and Kayan Mentarang National Park areas. Nevertheless, according to Balai Penelitian Tanah (Indonesian Soil Research Institute) (Balittanah 2009), the soil carbon percentage in the three *Rafflesia* habitats was classified as high to very high, while the nitrogen content was classified as medium to high.

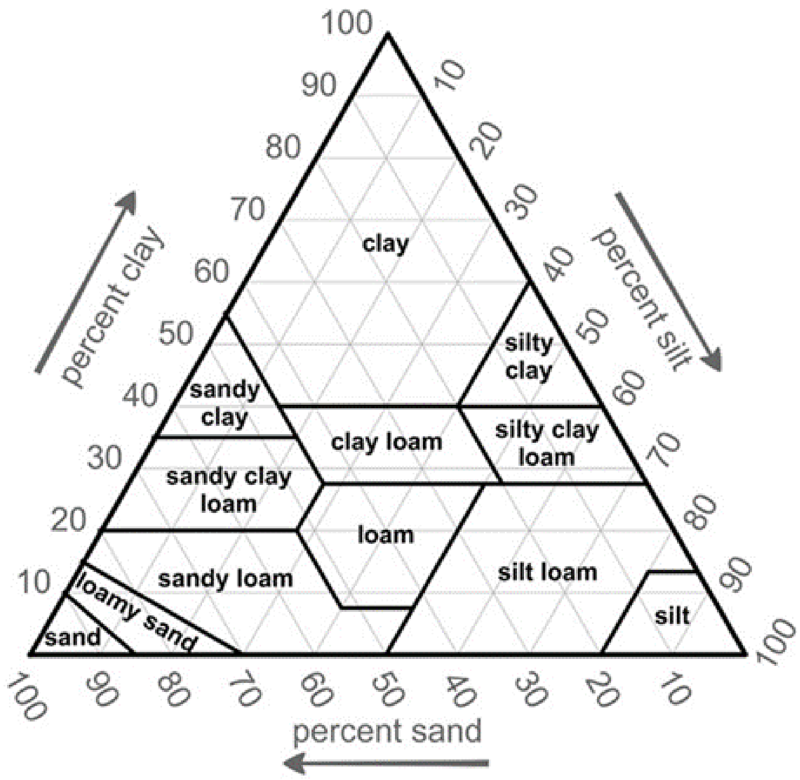

Figure 3. USDA Soil Texture Triangle (Groenendyk et al. 2015).

The ratio of carbon to nitrogen (C/N) in the soil can be influenced by various factors such as the type of vegetation, organic matter, and microbes. The optimum C/N ratio for plant growth is between 10-20. Our results showed that the C/N ratio in the three *Rafflesia* habitats was within this range, indicating that the soil has a higher level of decomposition and a faster release of nutrients (Kitayama et al. 1998; Eiland et al. 2001; Li et al. 2020)

In terms of phosphorus content, the Kayan Mentarang National Park site had the highest value at 96 ppm, which is considered very high, while the other two locations had lower levels with Palak Siring Kemumu at 13 ppm and Tempursari-Lumajang at 20 ppm (Table 2). In contrast, the Palak Siring Kemumu site had the highest base saturation at 99.3 %, followed by Tempursari-Lumajang at 86.99%, both of which were

Table 2. Soil Texture and Soil Chemical properties of three habitats of *Rafflesia* spp.

Location	Soil texture			Texture class	pH	C (%)	N (%)	C/N	P_2O_5	CEC	Sulphur	Base Saturation	Na
	Sand	Clay	Silt										
Tempursari, Lumajang	56	24	20	Sandy Clay loam	5.8	4.43	0.32	14	20	29.29	216	86.99	0.22
Palak Siring Kemumu	29	30	41	Clay Loam	6	7.67	0.69	11	13	28.54	58	99.3	0.19
Kayan Mentarang National Park	9	37	54	Silty clay loam	6	6.13	0.57	11	96	21.83	211	24.28	0.14

categorized as very high. The Kayan Mentarang National Park site had the lowest base saturation at 24.28%. Additionally, the cation exchange capacity (CEC) in all three habitats of *Rafflesia* spp. was high, with the highest value found in the Tempursari-Lumajang location at 29.29 me $100g^{-1}$, followed by Palak Siring Kemumu at 28.54 me $100g^{-1}$ and Kayan Mentarang National Park at 21.83 me $100g^{-1}$.

Moreover, the sulphur content varied among the three locations, with the highest content observed in the Tempursari-Lumajang location at 216, followed by Kayan Mentarang National Park at 211 and the lowest content in the Palak Siring Kemumu location at 58. The sodium content found at the low level. The highest sodium content was observed in the soil of the Palak Siring Kemumu location at 0.22 me $100g^{-1}$, followed by Tempursari-Lumajang at 0.19 me $100g^{-1}$ and Kayan Mentarang National Park at 0.14 me $100g^{-1}$. Sodium serves as an essential cation for plants, but excessive concentrations can be detrimental to plant growth, leading to root damage, compromised water and nutrient absorption, as well as disruptions in metabolic and cellular functions (Chang & Dregne 1955; Subbarao et al. 2003).

The soil texture classes of the habitat of four species *Rafflesia* also varied. The Tempursari-Lumajang location exhibited a sandy clay loam texture class, whereas the Palak Siring Kemumu location, situated in Sumatera, had a clay loam texture class. On the other hand, the Kayang Mentarang National Park location, located on the island of Borneo, had a silty clay loam texture class. These differences in soil texture can have implication for the existing of *Rafflesia* species and its host.

The soil texture affects the ability of soil to hold the available water and nutrients for plants thus directly affecting the plants growth. In Lumajang area, *R. zollingeriana* found in the sandy clay loam soil texture with higher percentage of sand in the soil. The previous studies reported that the *R. zollingeriana* found in Meru Betiri National Park, East Java found in soil with a high percentage of clay, sandy clay and sandy loam (Zuhud 1988; Laksana et al. 2018). Furthermore, previous study in the same national park, Maulidiyan et al. (2019) reported that *R. zollingeriana* occurred in an inclined rocky terrain, leading to the development of root buttresses in its host plant, *Tetrastigma* spp.

The two species of *Rafflesia*; *R. gadutensis* and *R. kemumu* found in clay loam, while the *R. pricei* found in silty clay loam soil texture. The silty clay loam soils tend to have moderate to high water-holding capacity and can retain water for relatively long periods of time compare to the sandy clay loam soil. The water uptake of *Rafflesia* is entirely dependent on its host plant, and therefore, the soil texture affects the water intake required for both the host plant and *Rafflesia*. The sandy clay loam, clay loam, and silty clay loam soil textures are considered have the ability to meet the water requirements of *Tetrastigma* and *Rafflesia*. Sandy clay loam soils are typically well-draining and can hold water for moderate periods, while clay loam soils, with their higher proportion of clay particles, have a higher water-holding capacity and are generally considered to be some of the most productive and versatile soils. Silty clay loam soils show a balance between water-holding capacity and drainage (Saxton et al. 1986; Andrenelli et al. 2016). Furthermore, *Rafflesia* required the soil texture that can support good drainage, which prevents waterlogging, root damage and *Rafflesia* decaying. The soil should able to hold onto sufficient moisture to meet the needs of the host and *Rafflesia*, but also allow excess water to drain away relatively quickly.

Calcium (Ca), magnesium (Mg), and potassium (K) are essential soil nutrients for the growth of plants including the host of *Rafflesia*:

Tetrastigma spp., although they are required in smaller quantities compared to other nutrients. The result showed that Potassium concentrations varied slightly among the study sites, ranging from 0.9 me 100g^{-1} to 1.79 me 100g^{-1} (figure 4). Calcium concentrations were highest at Palak Siring Kemumu, with a value of 22.92 me 100g^{-1}, followed by Tempursari, Lumajang, with a value of 17.98 me 100g^{-1}, and Kayan Mentarang National Park, with a value of only 1.73 me 100g^{-1}. The magnesium content also varied across the study sites, with the highest concentration found at Tempursari, Lumajang (6.16 me 100g^{-1}), and the lowest concentration found at Kayan Mentarang National Park.

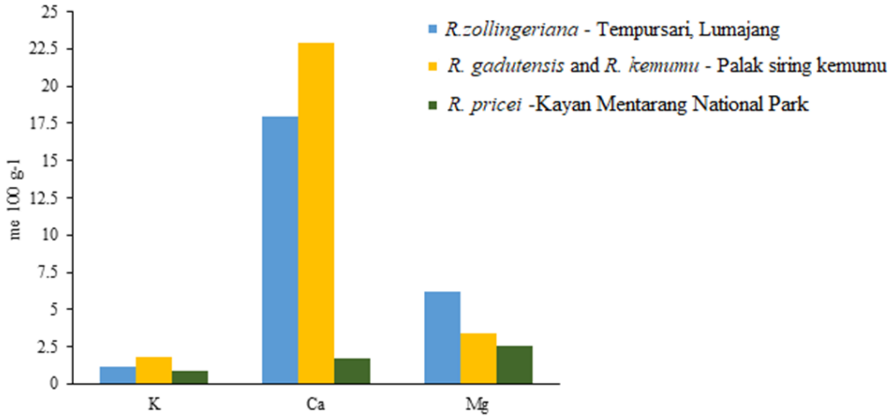

Figure 4. The Exchangeable Pottasium, Calsium and Magnesium content in the soil.

The Kayan Mentarang National Park, where *R. pricei* was discovered, exhibited the lowest base saturation. Low base saturation refers to a condition where the relative proportion of exchangeable base cations compared to the total soil cation exchange capacity (CEC), is relatively low (Juo et al. 1976; Chapman 2016). This indicates a reduced proportion of positively charged ions such as calcium, magnesium, potassium, and sodium in relation to the overall ion-holding capability of the soil. Moreover, the calcium content within the soil of Karang Mentarang was comparatively lower than that found in the other two locations. As a consequence of these distinctive soil attributes, the nutritional requirements of distinct *Tetrastigma* and *Rafflesia* species may exhibit variations contingent upon their respective geographic placements.

Variations in the edaphic characteristics were observed among populations of the same *Rafflesia* species. These differences can be due to multiple factors, including variations in elevation, geographical region, and the geological composition of soil formation. For instance, *R. zollingeriana* that found in Meru Betiri National Park, was encountered in soil of the Latosol type, characterized rich iron and aluminium oxide (Laksana et al. 2018). Previous study mentioned that this species tends to thrive in soils exhibiting a moderate to high Cation Exchange Capacity (CEC), high base saturation, and a high to very high concentration of potassium (K), sodium (Na), calcium (Ca), and magnesium (Mg). The carbon content of these soil ranges from moderate to high, while total nitrogen (N) content varies from low to moderate, accompanied by notably low phosphorus (P) levels (Zuhud 1988). The disparities in location and elevation contribute to variations in soil chemical character within the habitat of *R. zollingeriana* and its host plant. In Meru Betiri National Park, *R. zollingeriana* was found at elevations ranging from 0 to 300 meters above sea level (Zuhud 1988; Lestari et al. 2014), while in the Lumajang region of East Java, it was encountered at elevations of 450 meters above sea level (Lestari & Susatya 2022).

Edaphic variation also found in *R. gadutensis* habitat. Despite its presence in Palak Siring Kemumu, *R. gadutensis* also distributed within West Sumatera (Meijer 1984; Meijer 1997; Nais 2001; Rahma et al. 2017). Based on the observation of Akhriadi et al. (2010), the population of *R. gadutensis* could be found elevations ranging from 350 to 750 meters above sea level, specifically within old secondary rainforest in West Sumatera. Additionally, the presence of *R. gadutensis* populations have been recorded on the diminutive island of Mursala, proximate to Sibolga along the western coastline of North Sumatra (Mahyuni et al. 2016). Meanwhile, *R. kemumu* is a new species of *Rafflesia* found in the lowland forests of Bengkulu (Susatya et al. 2017) and there has been no study regarding the presence of *R. kemumu* in areas other than Bengkulu.

This study has confirmed that each species of *Rafflesia* requires a distinct set of soil characteristics in its edaphic habitat. Despite variations between the habitats studied, we observed that the soil pH was slightly acidic, while carbon and nitrogen levels ranged from moderate to high. These findings emphasize the essential significance of nutrient-rich soil and a resilient cation exchange capacity (CEC) in facilitating the growth of both the host plant and *Rafflesia*. As such, these pivotal factors must be considered in the both in situ and ex-situ conservation initiatives aimed at safeguarding and nurturing *Rafflesia* populations. By acknowledging and integrating these soil-related insights, we can enhance the efficacy and success of conservation efforts for these remarkable species.

In terms of in-situ conservation, environmental changes, such as soil compaction and erosion, can adversely affect the growth and survival of *Tetrastigma*, directly impacting *Rafflesia* as well. Given the high sensitivity of *Rafflesia* to environmental changes, especially when its host plant is compromised, it is necessary to maintain the original edaphic conditions of both the host and *Rafflesia* undisturbed.

Due to the fact that the selection of an appropriate host for *Rafflesia* is determined by intricate physiological compatibility rather than a single factor alone (Renjana et al. 2022), in terms if ex situ conservation, it becomes crucial to replicate all abiotic and biotic factors present in natural habitat of *Rafflesia* as closely as possible to improve host compatibility with *Rafflesia*, to enhance the success of *Rafflesia* propagation.

The soil characteristics from the three locations reflect fertile soil with an adequate supply of macronutrients for the growth of the host plant. Our ongoing study has indicated that the combination of soil, compost, and fermented bamboo leaves present the potential medium for the cultivation of *Tetrastigma*, providing fertile soil and a favorable texture. However, further investigation is required to determine the optimal proportions for this media composition.

In addition to soil composition, the water and nutrient requirements of the soil are critical factors influencing the growth of both *Tetrastigma* and *Rafflesia*. This correlation arises because the development of the *Rafflesia* bud intricately tied to the host plant vine, acquiring water and nutrients (Nais 2001; Iman et al. 2021). Consequently, more comprehensive studies are imperative to identify the specific essential nutrients essential for the growth and development of the *Rafflesia*. This detailed observation will contribute to valuable insights towards creating an optimal environment that supports the thriving coexistence of *Tetrastigma* and *Rafflesia*.

AUTHOR CONTRIBUTION

Conceptualisation, D.L, RI and F.A.S.; validation and data curation, F.A.S and D.L., writing—original draft preparation, F.A.S.; writing, review and editing, F.A.S, D.L and R.I; F.A.S and D.L are the main authors of this publication. All author has reviewed and approved the final draft of the manuscript for publication.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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