Avifauna in Logged-Over Forest of Upper Baleh, Sarawak

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ABSTRACT

Commercial logging is a major economic activity in the Upper Baleh catchment, Sarawak, so logged-over forest is the dominant forest type there. Avifauna survey was conducted in the logged-over forest of Upper Baleh in November 2015 as part of the Upper Baleh Heart of Borneo Expedition. The objective of the survey was to collect baseline data on the avifauna species that inhabit the study area, their conservation status and feeding guilds. Both mist-net and observation method were used. A total of 95 species of birds was recorded: 36 species via mist-nets and 69 species via observation. Little spiderhunter was the dominant species, accounting for 33% of mist-netted bird. Seven species are Totally Protected including six species of hornbills and a Great Argus Pheasant, while 18 other species are Protected under the Sarawak Wild Life Ordinance 1998. The majority of the birds are insectivorous (55.8%), foraging either at ground level (babblers), along the tree trunks or branches (woodpeckers) or at the canopy (flycatchers). Omnivorous birds, which feed on two or more types of diet, accounted for 48.4% of the avifauna species recorded and these include bulbuls and hornbills. The diverse community of bird, including the protected species, makes the area an attractive birding destination for visitors since now part of the catchment has been gazetted as a national park. Avifauna's role as pollinating and dispersing agent will help the logged-over forest to recover.

Keywords: conservation status, feeding guilds, Heart of Borneo, logging roads

INTRODUCTION

The Baleh River is a tributary of Rajang and has its origin in the Nieuwenhuis highlands that forms the border between Sarawak and Central Kalimantan. It has a large catchment area of 12,433 km^2 and contributes significantly to the Rajang River basin. For the Kenyah community of Long Singut who settled at Upper Baleh in the late 1960s, the river is the only way to go down to the main towns of Kapit and Sibu. Currently, the main economic activity affecting the forest and thus the habitats of wildlife in the Upper Baleh region is commercial logging. Shifting cultivation is generally confined to accessible areas next to the river and human settlement.

Commercial logging in Sarawak started soon after the Second World War (Aiken & Leigh, 1992) focusing mainly on peatswamp species. Strong demand for tropical timber and favourable government policies had accelerated the growth of the timber industry to become the mainstay of the economy with log production peaking at 19 Mm^3 in 1990 (Hon & Shibata, 2013). According to Bryan *et al*. (2013) about 364,489 km of logging roads were constructed in Sarawak, Sabah and Brunei between 1999 and 2009 alone, with Sarawak having the highest density of logging roads in Borneo (0.89 km/km^2). A major consequence of this logging activity in Sarawak is the reduction in intact forest, from 92,152 km^2 in 1973 to 18,161 km^2 in 2010 (Gaveau *et al*., 2014). These intact forests are likely to be logged or converted into other forms of designated land use since they are not legally protected.

In Sarawak, legal protection is given to 37 Totally Protected Areas (TPAs) equivalent to 483,682 hectares of land, which have been gazetted for biodiversity conservation and appreciation of nature (Sarawak Forest Department, 2017). These TPAs are the only land that can be considered safe from logging and cultivation. However, many TPAs do not have management presence and their boundaries are not clearly marked, so encroachment into these areas is a major concern (Gumal, 2007; Mohd Azlan & Lawes, 2011). In addition, the majority of TPAs in Sarawak are less than 10,000 km^2 in size and scattered throughout the State, with little regards for connectivity. This compromises their

capacity to sustain viable population of large animals with large home range (Mohd Azlan & Lawes, 2011).

Aware of the shortcomings of the current system of protected areas and conscious of the need to balance economic development and biodiversity conservation in Borneo, the government of Malaysia, Indonesia and Brunei agreed in February 2007 to commit funding and resources to promote sustainable development and conserve biodiversity under the Heart of Borneo (HoB) Initiative (http://wwf.panda.org). The Upper Baleh catchment is located in central Borneo and is part of the 22 million hectares under the HoB Initiative. The forest there is among the last to be logged circa 1990s, and also the least known in terms of its avifauna composition. Its avifauna has never been documented by earlier naturalists and explorers that put Borneo on the zoological map (Cranbrook & Leh, 1983; Smythies, 1999; Tuen, 2005).

Hence a mini scientific expedition to Upper Baleh was jointly organized by Universiti Malaysia Sarawak (UNIMAS), Sarawak Forestry Department (SFD) and World Wide Fund for Nature (WWF) in November 2015. One of the components of this expedition was a survey of avifauna with the objective to produce a checklist of the bird species inhabiting the logged-over forest of Upper Baleh, Kapit Division, Sarawak, including their conservation status and feeding guilds. The results of the survey are presented in this paper.

MATERIALS AND METHODS

Sampling Site

Avifauna sampling was carried out using mist-nets in the hill dipterocarp forest near Elite Honour Camp and by observation along the logging road heading east until the base of White Rock Mountain near Batu Tiban about 10 km from Indonesian Border. Elite Honour Camp (Figure 1) is one of the logging camps located on hill slope south of Baleh River, within a WTK logging concession area.

The mist-netting sites were located on a ridge about 300 meters above the sea level. The forest near the camp was relatively undisturbed because it was the water catchment for the camp while the 1000-meter site was logged about 10 years ago and comprised mainly of secondary growth forest. Active logging was being carried out in many parts of the concession area with the area near the base of White Rock Mountain being opened up for logging for the first time. The gaps created by falling timber trees and skid trails from earlier logging activity have reverted into secondary forests. Other secondary forest areas are fallow land, which is close to the river bank where it has been cleared for padi planting by the Long Singut Kenyah community.

Sampling Method

Mist-netting is a preferred method for bird survey when bird ringing is required or when more information is sought from each bird (Karr, 1981). Ten mist-nets were set up about 100 meters from Elite Honour Camp (Station 1, N01° 33' 32"; E114° 11' 9) and another 10 nets were set up about 1000 meters east the camp (Station 2, N01° 33' 35"; E114° 12' 7"). The mist-nets were operated between 6 am and 6 pm from 21-25 November 2015.

Observation was carried out opportunistically with the aid of binoculars (Nikon, 7x42) around the logging camp area and along the logging road from the camp to Long Singut in the east, then to the base of the White Rock Mountain where active logging is being carried out. The total distance of the logging road surveyed was 52 km

Figure 1. Map of Borneo showing location of Upper Baleh where the expedition was carried out (a) and avifauna sampling stations near Elite Honour Camp (b). △ Location of Elite Honour Camp.

and the elevation ranged from 220 to 760 meters above sea level. Birds were detected both via sighting and vocalisation; and species detected through vocalisation were recorded only if confirmed by sighting. Identification of feeding guilds followed Smythies (1999) and the naming of species followed Smythies (1999) and Phillipps (2014).

RESULTS

The combination of mist-net and observational method employed during this expedition yielded a total of 95 avifauna species (Table 1). Thirty-six species were recorded using mist-net while 69 were recorded using observational method. This list represents the first record of avifauna community for Upper Baleh catchment. Species such as Cattle Egret, Little Egret, Common Sandpiper, Grey Wagtail and Tree Sparrow (observed around the logging camp compound) are not strictly forest birds.

Of the 36 species caught using mist-net, the most abundant was Little Spiderhunter (54 individuals, 33%). In contrast, 11 species were caught only once, including Crested Goshawk, Plaintive Cuckoo, Black and White Bulbul, Chestnut-naped Forktail, Malaysian Blue Flycatcher and Long-billed Spiderhunter. Although the cumulative graph of species caught using mist-net (Figure 2) appeared to have reached a plateau, more species would have been added to the list had more sites been sampled and sampling period prolonged.

Eighteen of the species that we recorded are listed as Protected under the Sarawak Wild Life Protection Ordinance 1998. These include eagles (4), woodpeckers (3), egrets (2), Common Sandpiper, Rufous-backed Kingfisher, Imperial Pigeon, Garnet Pitta, Buffy Fish Owl, Hanging Parrot, White-rumped Shama, Chestnut-naped Forktail and Hill Myna. Seven of the species that we recorded are listed as Totally Protected under the same ordinance where these include six species of hornbills and the Great Argus Pheasant. All of the Totally Protected species were detected by observation only, which indicates the usefulness of this method for conservation assessment and thus, there is a need to train more students to be proficient in this method.

Three migrant species (Blue and White Flycatcher, Grey Wagtail and Cattle Egret) and three montane endemics species (Mountain Imperial Pigeon, Mountain Barbet and Little Cuckoo-Dove) were also identified during the observation. The egrets (Little Egret and Cattle Egret) were spotted flying up and down Baleh River. Only four of the species we recorded are endemic to Borneo, which are Mountain Barbet, Bornean Brown Barbet, Dusky Munia and Yellow-rumped Flowerpecker. None of the species recorded is categorized as Threatened according to IUCN Red List of Threatened Species 2018.

The majority of the birds are insectivorous (55.8%), foraging either at ground level (babblers), along the tree trunks or branches (woodpeckers) or at the canopy (flycatchers). A further 18.9% of the birds also include insects as their secondary diet. Omnivorous birds, which feed on two or more types of diet accounted for 48.4% of the avifauna species recorded and these include bulbuls and hornbills.
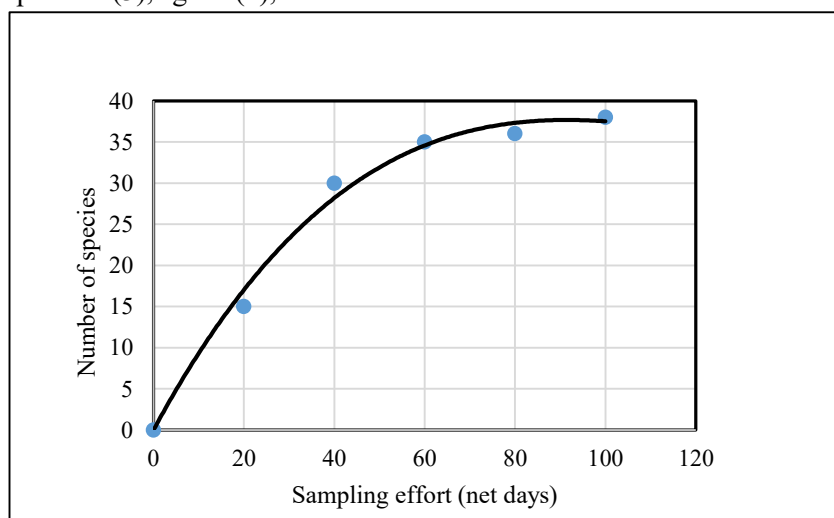

Figure 2. Cumulative graph of mist-netted birds recorded in Ulu Baleh in November 2015.

Table 1. Summary of avifauna species recorded in Ulu Baleh in November 2015 (Status: WPO = Wild Life Protection Ordinance 1998, IUCN = IUCN Red List of Threatened Species 2015, P = Protected, TP = Totally Protected, NA = not yet assessed, LC = Least Concern, NT = Near Threatened, Guild represent the main feeding guild of the bird, C = carnivore, F = frugivore, G = granivore, I = insectivore, N = nectarivore, O = omnivore).

| Family/Species | Conservation Status | | | Detection method | | Guild |
	WPO	IUCN	Residential	Mist-net	Observation	
Accipitridae						
Crested Goshawk (*Accipiter trivigatus*)	P	NA	Resident	1		C
Crested Serpent-eagle (*Spilornis cheela*)	P	LC			2	C
Black Eagle (*Ictinaetus malayensis*)	P	LC			1	C
Rufous-bellied Hawk-eagle (*Spizaetus cirrhatus*)	P	NA			1	C
Ardeidae						
Cattle Egret (*Bubulcus ibis*)	P	LC	Migrant		1	C, I
Little Egret (*Egretta garzetta*)	P	LC	Migrant & Resident		4	C, I
Scolopacidae						
Common Sandpiper (*Actitis hypoleucos*)	P	LC	Resident		1	C
Phasianidae						
Great Argus (*Argusianus argus*)	TP	NT	Resident		2	I, F
Columbidae						
Mountain Imperial Pigeon (*Ducula badia*)	P	NA	Montane resident		3	F
Emerald Dove (*Chalcophaps indica*)	-			7		F, G
Little Cuckoo-dove (*Macropygia ruficeps*)		NA	Submontane resident		2	F, G
Psittacidae						
Blue-crowned Hanging Parrot (*Loriculus galgulus*)	P	LC	Resident		2	F, I
Cuculidae						
Violet Cuckoo (*Chrysococcyx xanthorhynchus*)		LC	Resident		1	I, F
Plaintive Cuckoo (*Cacomantis merulinus*)	-	LC	Resident	1		I, F
Raffles's Malkoha (*Phaenicophaeus chlorophaeus*)	-	LC	Resident		4	I
Chestnut-breasted Malkoha (*Phaenicophaeus curvirostris*)			Resident		1	I
Lesser Coucal (*Centropus bengalensis*)	-	LC	Resident		1	I, O
Strigidae						
Buffy Fish-owl (*Ketupa ketupu*)	P	LC	Resident		1	C,
Apodidae						
Silver-rumped Needletail (*Rhaphidura leucopygialis*)			Resident		4	I
Trogonidae						
Red-naped Trogon (*Harpactes kasumba*)		LC	Resident		1	I
Alcedinidae						
Rufous-backed Kingfisher (*Ceyx rufidorsus*)	P	LC	Resident	4	1	I
Meropidae						
Red-bearded Bee-Eater (*Nyctyornis amictus*)	-		Resident		1	I
Bucerotidae						
White-crowned Hornbill (*Berenicornis comatus*)	TP	LC	Resident		1	F, O
Bushy-crested Hornbill (*Anorrhincus galeritus*)	TP	LC	Resident		9	F, O
Black Hornbill (*Anthracoceros malayanus*)	TP	NT	Resident		2	F, O
Oriental Pied Hornbill (*Anthrococeros albirostis*)	TP	LC	Resident		2	F, O
Rhinoceros Hornbill (*Buceros rhinoceros*)	TP	NT	Resident		1	F, O
Wreathed Hornbill (*Rhyticeros undulatus*)	TP		Resident		2	F, O
Megalaimidae						
Bornean Brown Barbet (*Calorhamphus fuliginosus*)	-	LC	Endemic		3	I, F
Red-crowned Barbet (*Megalaima rafflesii*)	-	NT	Resident		1	F
Red-throated Barbet (*Megalaima mystacophanos*)	-	NT	Resident		2	F, O
Yellow-crowned Barbet (*Megalaima henricii*)	-	NT	Resident, sub-montane		3	F, O
Blue-eared Barbet (*Megalaima australis*)	-	LC	Resident		2	F
Gold-whiskered Barbet (*Megalaima chrysopogon*)	-	LC	Resident		1	F, O
Mountain Barbet (*Megalaima monticola*)	-	LC	Montane endemic		1	F, I
Picidae						
Rufous Piculet (*Sasia abnormis*)	P	LC	Resident	3		I

Table 1. Continued.

Family/Species	Conservation Status			Detection method		Guild
	WPO	IUCN	Residential	Mist-net	Observation	
Picidae						
Crimson-winged Woodpecker (*Picus puniceus*)	P	LC	Resident		1	I
Maroon Woodpecker (*Blythipicus rubiginosus*)	P	LC	Resident	3		I
Pittidae						
Garnet Pitta (*Pitta granatina*)	P	LC	Resident		1	I
Campephagidae						
Black-winged Flycatcher-shrike (*Hemipus hirundinaceus*)	-	LC	Resident		2	I
Bar-winged Flycatcher-shrike (*Hemipus picatus*)	-	LC	Montane Resident		1	I
Eurylaimidae						
Green Broadbill (*Calyptomena viridis*)	-	NT	Resident	2		F
Black-and-yellow Broadbill (*Eurylaimus ochromalus*)	-	NT	Resident		1	I
Banded Broadbill (*Eurylaimus javanicus*)	-	LC	Resident		1	I
Hirundinidae						
Pacific Swallow (*Hirundo tahitica*)	-	LC	Resident		23	I
Motacillidae						
Grey Wagtail (*Motacilla cinerea*)	-	LC	Migrant		1	I
Pycnonotidae						
Buff-vented Bulbul (*Iole olivacea*)	-		Resident	3	2	F, I
Finsch's Bulbul (*Criniger finschii*)	-		Submontane Resident		2	I, F
Hairy-backed Bulbul (*Tricholestes criniger*)	-		Resident	6		I, F
Black-headed Bulbul (*Pycnonotus atriceps*)	-	LC	Resident		2	F, I
Olive-winged Bulbul (*Pycnonotus plumosus*)	-	LC	Resident		1	I, F
Red-eyed Bulbul (*Pycnonotus brunneus*)	-	LC	Resident	2		I, F
Scaly-breasted Bulbul (*Pycnonotus squamatus*)	-		Submontane Resident		2	F, I
Spectacled Bulbul (*Pycnonotus erythropthalmos*)	-	LC	Resident	8		I, F
Grey-bellied Bulbul (*Pycnonotus cyaniventris*)	-		Resident		1	F, I
Grey-cheeked Bulbul (*Alophoixus bres*)	-	LC	Resident	3		I, F
Yellow-bellied Bulbul (*Alophoixus phaeocephalus*)	-	LC	Resident	8		I, F
Black and White Bulbul (*Pycnonotus melanoleucos*)	-	LC	Resident	1		F, I
Turdidae						
Oriental Magpie-Robin (*Copyschus saularis*)	-	LC	Resident		1	I
White-rumped Shama (*Copyschus malabaricus*)	P	LC	Resident	4	2	I
Chestnut-naped Forktail (*Enicurus ruficapillus*)	P	LC	Resident	1		I
Timaliidae						
Black-capped Babbler (*Pellorneum capistratum*)	-	LC	Resident		2	I
Ferruginous Babbler (*Trichastoma bicolor*)	-	LC	Resident	2	1	I
Short-tailed Babbler (*Malacocincla malaccense*)	-	NT	Resident	3	1	I
Scaly-crowned Babbler (*Malacopteron cinereum*)	-	LC	Resident	1		I
Sooty-capped Babbler (*Malacopteron affine*)	-	LC	Resident	1		I
Rufous-crowned Babbler (*Malacopteron magnum*)	-	LC	Resident	2		I
Striped Tit Babbler (*Macronous gularis*)	-	LC	Resident		3	I
Fluffy-backed Tit Babbler (*Macronous ptilosus*)	-	NT	Resident	4	2	I
Black-throated Babbler (*Stachyris nigricollis*)	-	NT	Resident	3		I
Chestnut-winged Babbler (*Stachyris erythroptera*)	-	LC	Resident	2	2	I
Chestnut-rumped Babbler (*Stachyris maculata*)	-	LC	Resident	5		I
Rufous-fronted Babbler (*Stachyris rufifrons*)	-	LC	Resident		1	I
Grey-headed Babbler (*Stachyris poliocephala*)	-	LC	Resident	7		I
Eupetidae						
Brown Fulvetta (*Alcippe brunneicauda*)	-	NA	Resident		2	I, F
Pardalotidae						
Rufous-tailed Tailorbird (*Orthotomus sericeus*)	-	LC	Resident	1	1	I
Muscicapidae						
Pied Fantail (*Rhipidura javanica*)	-	LC	Resident		2	I
Blue-and-white Flycatcher (*Cyanoptila cyanomelana*)	-	LC	Migrant			I, F
Malaysian Blue Flycatcher (*Cyornis turcosus*)	-	LC	Resident	1	1	I
Rufous-winged Philentoma (*Philentoma pyrhoptera*)	-	LC	Resident	1		I

Table 1. Continued.

Family/Species	Conservation Status			Detection method		Guild
	WPO	IUCN	Residential	Mist-net	Observation	
Muscicapidae						
Grey-headed Canary Flycatcher (*Culicicapa ceylonensis*)		LC	Resident		1	I
Dicaeidae						
Yellow-breasted Flowerpecker (*Prionochilus maculates*)	-	LC	Resident	4		I, F
Yellow-rumped Flowerpecker (*Prionochilus xyanthopygius)*	-		Endemic	8		N, I, F
Nectariniidae						
Purple-naped Sunbird (*Hyprogramma hypogrammicum*)	-	LC	Resident	4		N, I, F
Plain Sunbird (*Anthreptes simplex*)	-	LC	Resident	1	1	N, I, F
Temminck's Sunbird (*Aethopyga temminckii*)	-	LC	Resident		3	N, I
Little Spiderhunter (*Arachnothera longirostra*)	-	LC	Resident	54		N, I
Long-billed Spiderhunter (*Arachnothera robusta*)	-	LC	Resident	1		N, I
Thick-billed Spiderhunter (*Arachnothera crassirostris*)	-	LC	Resident		1	N, I
Sturnidae						
Hill Myna (*Gracula religiosa*)	P	LC	Resident		2	F, O
Passeridae						
Eurasian Tree Sparrow (*Passer montanus*)	-	LC	Resident		5	G
Estrildidae						
Dusky Munia (*Lonchura fuscans*)	-	LC	Endemic		4	G, I, F
Black-headed Munia (*Lonchura malacca*)	-	LC	Resident		7	G
Irenidae						
Asian Fairy Bluebird (*Irena puella*)	-	LC	Resident	2	2	F, I
Corvidae						
Slender-billed Crow (*Corvus enca*)	-	LC	Resident		8	I, F

DISCUSSION

The main limitation of this study is that the survey covers a very small area (52-km of logging roads) and only two mist-netting sites over a very short time (five days). However we have tried to increase the efficiency of our survey by combining both observational and mist-netting method leading to a haul of 95 species: 69 detected by observation and 36 using mist-net. Whitman *et al.* (1997) also reported more species recorded using observational method compared to mist-nets. Our data is also probably biased towards birds that are still common and easily detected due to their active and noisy behaviour. The bird list reported here represents the first record for Upper Baleh where this could provide a snapshot of what tourists expect to see if they come to this area as well as a reference for future work in the area.

Logging and shifting cultivation was still going on in the Upper Baleh area during the time of the expedition. Selective logging such as the one practiced in Upper Baleh catchment can remove about 54% of trees greater than 30 cm diameter at breast height (dbh) and a further 13% are damaged incidentally due to falling timber trees, construction of skid trails and logging roads (Bennett & Gumal, 2001) while Berry

et al. (2010) reported 21% of trees were removed by direct logging and another 32% by collateral damage associated with logging operation. Selective logging affects all bird species within the forest through opening of the canopy, intrusion of sunlight, drying, temperature increase, soil erosion, reduction of leaf litter, and effects on the invertebrates, flower and fruit production and changes in the predator community. The effect would be more devastating especially if the timber trees and other affected plants are food sources, nesting sites or place of refuge for Threatened or Protected bird species (Johns, 1988).

Lambert (1992) found that the abundance of some species of birds increased in selectively logged forests in Peninsular Malaysia compared to unlogged forests, while the abundance of other species decreased. An observational study conducted in Seram Island, Indonesia found that the avifauna diversity was lower in logged compared to unlogged forest (Marsden 1998). Repeatedly logged forest retained about 75% bird species richness of unlogged forest (Edwards *et al.*, 2011).

The effect of logging on avifauna communities seems to depend on species traits such as size of birds and their feeding heights and guilds, and

logging practices (Cleary *et al*., 2007; Burivalova *et al*., 2015; Hamer *et al*., 2015). Large avifauna species seemed to be particularly vulnerable because of their greater metabolic needs and habitat range which are disrupted by logging activities (Constantini *et al*., 2016). Logging appeared to have the strongest negative effect on the abundance of hornbills (Cleary *et al*., 2007; Naniwadekar *et al*., 2015) perhaps because of selective removal of large trees which are more likely to have tree holes that are used by birds for nesting. This can have negative implications on forest regeneration since hornbills are one of the most effective agents of seed dispersal (Kitamura, 2011). Six species of hornbills (out of eight species present in Borneo) were recorded in the Upper Baleh study area, suggesting either the logging intensity practiced by the company is not as severe as in other areas or the effects of logging are not complete since logging operation is still on-going. Insectivorous birds are particularly vulnerable to habitat disturbance (Sekercioğlu *et al*., 2002, Gray *et al*., 2007), perhaps because they constitute the majority of feeding guilds. However, this seem to depend on their trophic level, as well as their body size and foraging heights. Studies with babblers showed that large ground-feeding species occupying high trophic positions were more adversely affected than small understory-feeders with lower trophic positions (Hamer *et al*., 2015). This is attributed to a change in leaf litter arthropod composition (Burghouts *et al*., 1992), which in turn affect the abundance of their predators. In Upper Baleh, 53/95 avifauna species (55.7%) are insectivorous and a further 18 species that are categorized as omnivorous have insects in their diet. The predominance of insectivores in primary forest and agroforest was also reported recently by Attiqqah *et al*. (2017).

Logging roads can have a devastating impact on ecologically specialized species that cannot adapt to disturbances in their ecosystem (Laurance *et al.,* 2009, Edwards *et al*., 2017). Logging roads open up the previously inaccessible areas to hunters making game animals especially more vulnerable. For example, an estimated 29,000 kg of wild meat was consumed by a population of 167 workers, mainly Iban, at Nanga Gaat logging camp located within the Baleh catchment, of which bearded pig constitute 71.4% (Dahaban *et al*., 1996). Although birds are not normally the target, sometimes they are trapped for food as

well as for pet, especially Blue-crowned Hanging Parrots. A few parrots were kept as pets in cages by the Long Singut community within the Upper Baleh study area. In addition, the logging roads and skid trails do not only remove trees but also disturb the soil where these may have either positive impacts (exposes ground dwelling organisms e.g. worms, upon which bird feeds on) or negative impacts (destroys food source, nests and refuge for ground dwelling birds).

Besides logging, there was also conversion of forests into rice fields by the villagers of Long Singut. The process of land clearing for rice cultivation by the community is devastating for wildlife because it involves not only felling of trees but burning of the dried vegetation before planting can take place. The area affected at any one time is small, generally not more than one hectare per family, and after harvesting the land is usually left to grow back into secondary forest. Other threat includes the carnivorous pets (cats and dogs) owned by the logging community as well as the local community of Long Singut; these pets may harass wildlife, including birds.

Avifauna plays an important role in maintaining ecological balance through the services they provided, some of which can directly or indirectly benefit humans. These services include flower pollination and seed dispersers and the birds are therefore serve as important agents in the forest regeneration and recovery from disturbance. Other birds such as owls, hawks and eagles that feed on small vertebrates and the insectivorous birds such as babblers play an important role in biological pest control.

The ecosystem and biodiversity of Upper Baleh may be spared from further damage with the implementation of the HoB programs and initiatives, which may include gazetting the study area as a national park. The firm stance of Sarawak State government against illegal logging (New Straits Times, 2014), including by not issuing new timber license, will eventually allow the ecosystem to recover. Both the HoB Initiative and hydroelectric power dam further downstream has put the area under greater scrutiny from conservation-minded organization. This scrutiny and the greater awareness due to publicity and findings of the expedition has prompted the Sarawak Government to recently announce the gazettement of 66,721 ha of Upper Baleh as a national park (Sarawak Forest Department, 2018). The small Kenyah community in Long

Singut are not expected to cause further damage to the ecosystem if they are given alternative means of livelihood under the HoB Initiative or better employment opportunities with the power supply company.

CONCLUSION

A total of 95 species were recorded during the expedition, including four endemic, seven Totally Protected and 18 Protected species. Insectivores and omnivores are the major feeding guilds of these birds. This diverse community of bird, including the presence of protected and colourful species, makes the area an attractive birding destination. Avifauna's role as pollinating and dispersing agent will help the logged and cultivated forest to recover.

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REFERENCES

Aiken, S.R. & Leigh, C.H. (1992). *Vanishing rain forests: the ecological transition in Malaysia*. Oxford, UK: Clarendon Press.

Attiqqah, F.S., Mohd-Azlan, J., Khatijah, I., Sing-Tyan, P., Rahah, M.Y., Mohd Hasri Al-Hafiz, H., Isa, S. & Tuen, A.A. (2017). Diversity and functional guild of understorey birds in different types of forest in Ulu Baram, Sarawak. Paper presented at UNIMAS Silver Jubilee Conference 2017, Pullman Hotel, Kuching.

Bennett, E.L. & Gumal, M.T. (2001). The interrelationships of commercial logging, hunting and wildlife in Sarawak. *The cutting edge, conserving wildlife in logged forests*. New York, USA: Columbia University Press. Pp 359-374.

Berry, N.J., Phillips, O.L., Lewis, S.L., Hill, J.K., Edwards, D.P., Tawatao, N.B. & Ong, R.C. (2010). The high value of logged tropical forests: lessons from northern Borneo. *Biodiversity and Conservation*, 19(4): 985-997.

Bryan, J.E., Shearman, P.L., Asner, G.P., Knapp, D.E., Aoro, G. & Lokes, B. (2013). Extreme differences in forest degradation in Borneo: comparing practices in Sarawak, Sabah, and Brunei. *PLoS ONE* 8(7): e69679.

Burghouts, T., Ernsting, G., Korthals, G. & De Vries, T. (1992). Litterfall, leaf litter decomposition and litter invertebrates in primary and selectively logged dipterocarp forest in Sabah, Malaysia. *Philosophical Transactions of the Royal Society London B,* 335(1275): 407-416.

Burivalova, Z., Lee, T.M., Giam, X., Şekercioğlu, Ç.H., Wilcove, D.S. & Koh, L.P. (2015). Avian responses to selective logging shaped by species traits and logging practices. *Proceedings of the Royal Society B,* 282: 20150164.

Cleary, D., Boyle, T., Setyawati, T., Anggraeni, C., Van Loon, E. & Menken, S. (2007). Bird species and traits associated with logged and unlogged forest in Borneo. *Ecological Applications,* 17(4): 1184-1197.

Cranbrook, Earl of. & Leh, C.M.U. (1983). A history of zoology in Sarawak. *Sarawak Museum Journal* XXXII, 53: 15-33.

Constantini, D., Edwards, D.P. & Simons, M.J.P. (2016). Life after logging in tropical forests of Borneo: a meta-analysis. *Biological Conservation*, 196: 182-188.

Dahaban, Z., Nordin, M. & Bennett, E.L. (1996). Immediate effects on wildlife of selective logging in a hill dipterocarp forest in Sarawak: mammals. *In* Edwards, D.S., Booth, W.E. & Choy, S.C. (Eds.). Tropical Rainforest Research – Current Issues. *Monographiae Biologicae*, Vol 74. Springer, Dordrecht.

Edwards, D.P., Larsen, T.H., Docherty, T.D., Ansell, F.A., Hsu, W.W., Derhé, M.A. & Wilcove, D.S. (2011). Degraded lands worth protecting: the biological importance of Southeast Asia's repeatedly logged forests. *Proceedings of the Royal Society of London B:* 278(1702): 82-90.

Edwards, F.A., Finan, J., Graham, L.K., Larsen, T.H., Wilcove, D.S., Hsu, W.W., Chey, V.K. & Hamer, K.C. (2017). The impact of logging roads on dung beetle assemblages in a tropical rainforest reserve. *Biological Conservation*, 205: 85-92.

Gray, M.A., Baldauf, S.L., Mayhew, P.J. & Hill, J.K. (2007). The response of avian feeding guilds to tropical forest disturbance. *Conservation Biology*, 21:133-141.

Gaveau, D.L.A., Sloan, S., Molidena, E., Yaen, H., Sheil, D., Abram, N.K., Ancrenac, M., Nasi, R., Quinones, M., Wielaard, N. & Meijjaard, E. (2014). Four decades of forest persistence, clearance and logging on Borneo. *PLoS ONE,* 9(7): e101654.

Gumal, M. (2007). Keynote address: TPA management and communities: Conserving totally protected areas with rural communities living in and around these areas. *Proceedings of the 8th Hornbill Workshop on Protected Areas and Biodiversity Conservation*. Pp 226-236.

Hamer, K.C., Newton, R.J., Edwards, F.A., Benedick, S., Bottrell, S.H. & Edwards, D.P. (2015). Impacts of selective logging on insectivorous birds in Borneo: the importance of trophic position, body size and foraging height. *Biological Conservation*, 188: 82-88.

Hon, J. & Shibata, S. (2013). A review on land use in the Malaysian State of Sarawak, Borneo and recommendations for wildlife conservation inside production forest environment. *Borneo Journal of Resource Science and Technology*, 3(2): 22-35.

Johns, A. (1988). Effects of "selective" timber extraction on rain forest structure and composition and some consequences for frugivores and folivores. *Biotropica,* 20(1): 31-37.

Karr, J.R. (1981). Surveying birds with mist nets. *Studies in Avian Biology*, 6: 62-67.

Kitamura, S. (2011). Frugivory and seed dispersal by hornbills (Bucerotidae) in tropical forest. *Acta Oecologia*, 37(6): 531-541.

Lambert, F.R. (1992). The consequences of selective logging for Bornean lowland forest birds. *Philosophical Transactions of the Royal Society London B*, 335(1275): 443-457.

Laurance, W.F., Goosem, M. & Laurance, S.G. (2009). Impacts of roads and linear clearings on tropical forests. *Trends in Ecology & Evolution*, 24(12), 659-669.

Marsden, S.J. (1998). Changes in bird abundance following selective logging on Seram, Indonesia. *Conservation Biology*, 12(3): 605-611.

Mohd Azlan, J. & Lawes, M.J. (2011). The efficacy of protected areas and future challenges for wildlife conservation in Sarawak. *In* Mokhtar, M. & Abdul Halim, J.C. (Eds.), *RIMBA2: Regional Sustainable Development in Malaysia and Australia*. Bangi, Malaysia: Lestari. Pp 136-146.

Naniwadekar, R., Shukla, U., Isvaran, K. & Datta, A. (2015). Reduced hornbill abundance associated with low seed arrival and altered recruitment in a hunted and logged tropical forest. *PLoS ONE*, 10(3), e0120062.

New Straits Times (20 November 2014). Tougher laws needed to fight illegal logging. https://www.nst.com.my/news/2015/09. Downloaded on 31 July 2018.

Phillipps, Q. (2014). *Phillipps' Field Guide to the Birds of Borneo*. Oxford: John Beaufoy Publishing.

Sarawak Forest Department (2018). *http://www.forestry.sarawak.gov.my/page-0-257-1012-National-Parks.html*. Downloaded on 31 July 2018.

Şekercioḡlu, Ç.H., Ehrlich, P.R., Daily, G.C., Aygen, D., Goehring, D. & Sandí, R.F. (2002). Disappearance of insectivorous birds from tropical forest fragments. *Proceedings of the National Academy of Sciences*, 99(1): 263-267.

Smythies, B.E. (1999). *Birds of Borneo*. 4th edition. Kota Kinabalu, Malaysia: Natural History Publications.

Tuen, A.A. (2005). A fauna study of the Rajang River Basin. *Proceedings of the 7th Hornbill Conference, Kuching, 2005.* Pp 115-134.

Whitman, A.A., Hagan III, J.M. & Brokaw, N.V. (1997). A comparison of two bird survey techniques used in a subtropical forest. *Condor*, 99: 955-965.

Tree Stands and Liana Community in Royal Belum State Park, Malaysia

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ABSTRACT

The diversity of lianas and trees were studied in five study sites of 100 x 20 m within the Royal Belum State Park, Malaysia with a view to provide baseline information on their incidence, taxonomy and ecological distributions. The sites include Sungai Kejar, Sungai Papan, Sungai Papan 2, Teluk Gopal and Sungai Kooi with at least 1000 m apart. These plots were further sub-divided into five sub-plots of 20 x 20 m each. Lianas with a diameter at breast height (dbh) ≥ 1 cm and trees with dbh ≥ 10 cm were identified and frequencies of occurrence were determined. Lianas comprising 92 species from 23 families while trees comprising 221 species and 48 families were enumerated. Annonaceae was the richest family of lianas and trees (19 species and 23 species respectively). *Connarus* (Connaraceae) and *Spatholobus* (Fabaceae) had the highest number of lianas (six species) whilst *Syzygium* (Myrtaceae) had the highest number of trees (11 species). There are significant differences in all the diversity indices among the study sites, except between Sungai Papan and Teluk Gopal which were the richest and most diverse in liana species. These two sites also showed high similarity index in liana species (0.50) followed by Sungai Kejar and Sungai Papan 2 (0.37). Sungai Kejar was however observed to have the highest tree species richness. These study sites could be described as very rich with a high diversity of lianas and trees. Although, it is richer in trees than lianas which means that the level of disturbance of the park is very low.

Keywords: Annonaceae, *Connarus,* forests, lianas diversity, Perak, *Syzygium*

INTRODUCTION

Tropical forests are important carbon pools, comprising approximately 40% of terrestrial carbon storage (Dixon *et al.*, 1994). None of that matters because deforestation still going rampant and the quality of habitable spaces turned plummet (Potter, 1999). Wildlife creatures especially, are threatened by the scenario and have always been in great jeopardy. The intact forest, Royal Belum State Park, is recognizable for the pristine jungle, hosting innumerable floras and faunas. The excessive beauty of the forest gives protection to wild animals like tigers, elephants etc. (Khairil *et al.,* 2012). Moreover, a sparse canopy covers wholly shade everything beneath as promoting a nature of complexity and essential for wildlife interaction.

Lianas are woody climber species that are very abundant in tropical forests forming about 45% of the total woody plants population in such forests (Addo-Fordjour *et al.*, 2008; Schnitzer & Bongers, 2011). Liana and tree exhibit mutual relationship, in which liana benefiting more than its counterpart. This invariably means that lianas are trees competitors which disrupt their regeneration processes (Tobin *et al.,* 2012). Over the decades, the studies on trees are ubiquitous and researchers mainly pinpointed tree behavior in terms of ecology, functions and benefits. On the contrary, the ecology of lianas and their distribution in tropical forests are scantily perceived and became a lesser known subject amongst researchers (Addo-Fordjour *et al.,* 2016). Unknowingly, lianas are branded as a plant habit that only contribute nuisance to the forest ecosystem. Though they are very useful in the proper functioning of tropical ecosystems, their abundance in such forests is disastrous to native trees (Schnitzer *et al.,* 2000; Pérez-salicrup, 2001; Ingwell *et al.,* 2010; Putz, 2012). The misconception, however, weighed our team

to uplift the information on the occurrence of lianas alongside trees and to list important and endangered species in Royal Belum State Park. This will provide an inventory of species that will be useful for future researchers.

MATERIALS AND METHODS

Study Site

This study was carried out at the Royal Belum State Park, Perak, Malaysia (Figure 1). This is a UNESCO World heritage site covering an area of 117,500 ha and straddling the northern,

undisturbed and pristine lowland dipterocarp, hill dipterocarp and lower montane forests (up to about 1,533 m above sea level) of northern Peninsular Malaysia. This forms the northern and strategic component of the Central Forest Spine (CFS). It is considered as one of the oldest protected park that is undisturbed in Peninsular Malaysia. This park has been referred to as the biodiversity hotspot in Malaysia hosting diverse ecosystems and habitats for several flora and fauna species in which many of them are endemic, rare, vulnerable or otherwise threatened in Malaysia and the region.
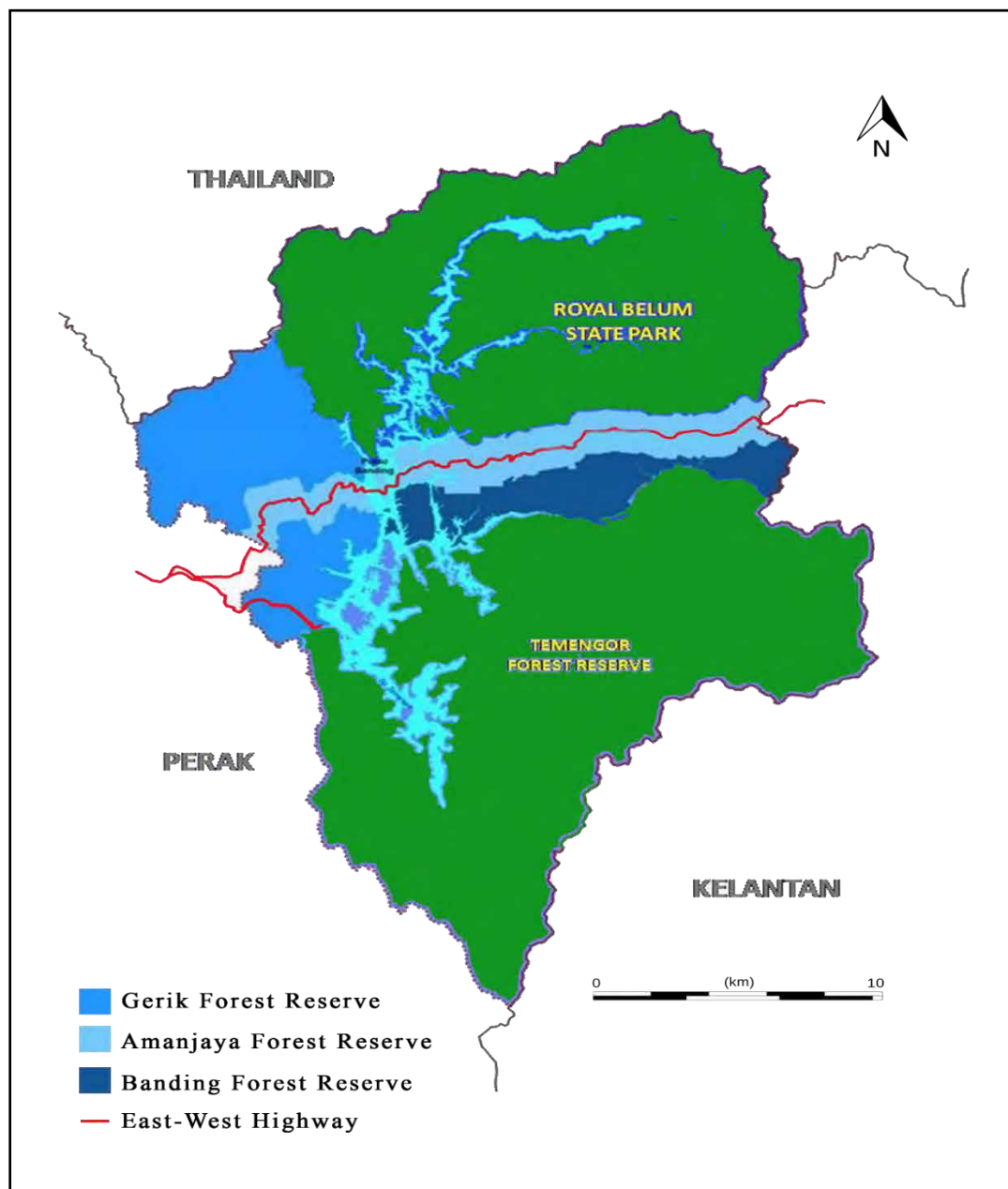

Figure 1. The map of Belum-Temengor Forest Complex (Redrawn from: https://mnshornbillvolunteerprogramme.wordpress.com/about/).

Sampling Procedure

The occurrence of liana and trees were enumerated in five study sites within the park, namely; Sungai Kejar, Sungai Papan, Sungai Papan 2, Teluk Gopal and Sungai Kooi (Table 1). For each site, plots were randomly established with the size of 100 x 20 m (0.2 ha). Subsequently, these plots were further divided into five subplots (20 m x 20 m). Within the subplots, lianas with dbh $\geq$ 1 cm and trees with dbh $\geq$ 10 cm were identified. All processed specimens were kept in the Herbarium of Universiti Sains Malaysia, Pulau Pinang for further reference. Unidentified species were recorded by physical appearances such as leaf, flowers and fruits for further recognition by referring to the books and forest manual provided (Ng, 1978, 1989; Whitmore,1983a,b; Kiew *et al.*, 2010, 2011, 2012).

Table 1. Geographical coordinate of sampling sites.

Location	Latitude (N)	Longitude (E)	Elevation (m)
Sungai Kooi	05° 37' 40.6"	101° 26' 40.8"	540
Sungai Papan	05° 37' 40.6"	101° 24' 10.3"	290
Sungai Papan 2	05° 37' 23.8"	101° 24' 39.0"	405
Sungai Kejar	05° 48' 06.2"	101° 25' 33.4"	514
Teluk Gopal	05° 36' 37.7"	101° 23' 28.4"	360

Statistical Analysis

The lianas and trees diversity indices such as Shannon index, Simpson index and Evenness index were quantified for each study site using PAST software (Rahmad & Akomolafe, 2018). Incidence-based rarefaction-extrapolation analysis, which is a non-asymptotic species richness evaluator, was carried out to estimate the species richness of lianas and trees species in the study sites using the twenty-five sample size.

A significant difference in species richness between the study sites was determined using confidence intervals, constructed by 100 bootstrap replicates (Addo-Fordjour *et al.,* 2016). Software called iNEXT (online version) was used for this (Chao *et al.,* 2016). If the confidence intervals of the curves do not overlap, estimates of species richness are regarded as significantly different. However, if the confidence intervals of the curves overlap, then the estimates of species richness are not significantly different.

Analysis of similarity is a non-dependent analysis of the sample size as the indication of the presence/absence data for species in a community are particularly used (Krebs, 1989). Range of similarity commences from 0.0 (least similar) to 1.0 (highly similar). Modified Morisita's Similarity was preferably used due to its independence on the sample size. Hence, the unweighted pair-group method using arithmetic averages (UPGMA) was performed as the clustering method (Romesburg, 1984). Multivariate Statistical Package (MVSP) version 3.22, Kovach Computing Services coordinated the analysis.

RESULTS

Diversity, Richness and Similarities of Liana Species Among Study Sites

In all study sites, 92 species of lianas belonging to 23 families were identified (S1). The largest liana family was Annonaceae (19 species) followed by Connaraceae (11 species) and Fabaceae (11 species). Annonaceae, which was the largest family, was composed of nine genera followed by Apocynaceae with four genera and Connaraceae with three genera (S1). In terms of a number of species, the richest genera are *Connarus* (six species), *Spatholobus* (six species) and *Strychnos* (five species). For each study site, Sungai Papan has the highest recorded species (39 species) followed by Teluk Gopal (38 species) and Sungai Kejar (37 species). Sungai Papan and Teluk Gopal also have the highest diversity indices compared with others (Table 2). There was overlap in the confidence intervals of the rarefied and extrapolated species richness curves of all the sites. Sungai Papan and Teluk Gopal had the highest species richness which was not significantly different (Figure 2).

Table 2. Diversity indices of liana species in the study sites.

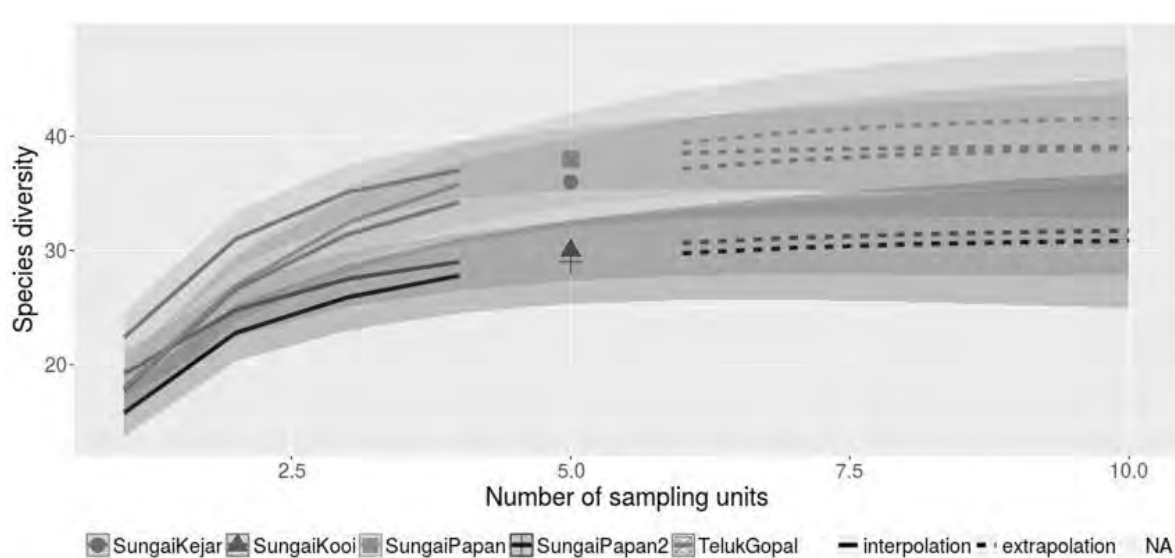| Diversity Indices	Sungai Kejar	Sungai Papan	Sungai Papan 2	Teluk Gopal	Sungai Kooi
Simpson Index	0.9667	0.9671	0.9603	0.9698	0.9615
Shannon Index	3.493	3.526	3.297	3.571	3.326
Evenness Index	0.889	0.872	0.901	0.912	0.898
Fisher alpha	22.5	24.72	16.69	20.49	15.27
Rarefied and extrapolated species richness	36	39	29	39	30

Figure 2. Incidence based rarefaction and extrapolation curves for species richness (including confidence intervals) of liana species. Solid lines represent rarefaction curves while dashed lines represent extrapolation curves. Each dot stands for the estimated species richness. The shadow of each curve represents the confidence interval.

Analysis of species similarity displays the cluster analysis of liana community in different sites (Figure 3). The highest value was indicated between Sungai Papan and Teluk Gopal (0.50) which implies the highest species similarity in both study sites, followed by Sungai Kejar and Sungai Papan 2 (0.37). Whilst, the comparison between Node 1 (consisting of Teluk Gopal and Sungai Papan) and Sungai Kooi read the second highest at 0.34. Notwithstanding, the lowest value was denoted between Node 2 (consisting of Sungai Kooi, Teluk Gopal and Sungai Papan) and Node 3 (consisting of Sungai Papan 2 and Sungai Kejar) at 0.36, which suggests the lowest species similarity recorded between them.

Diversity, Richness and Similarities of Tree Species Between Study Sites

Results showed that 221 trees species belonging to 48 families were identified in all the sites (S2). The families with the high number of species include Annonaceae (23 species, 16 genera) followed by Euphorbiaceae (22 species, 12 genera) and Meliaceae (18 species, eight genera). The richest genus was *Syzygium* (11 species) followed by *Dacryodes* (seven species) and *Shorea* (six species). Sungai Kejar was observed to have the highest tree species richness and diversity while Sungai Kooi had the lowest (Table 3). However, all the study sites had the same even distribution of tree species.
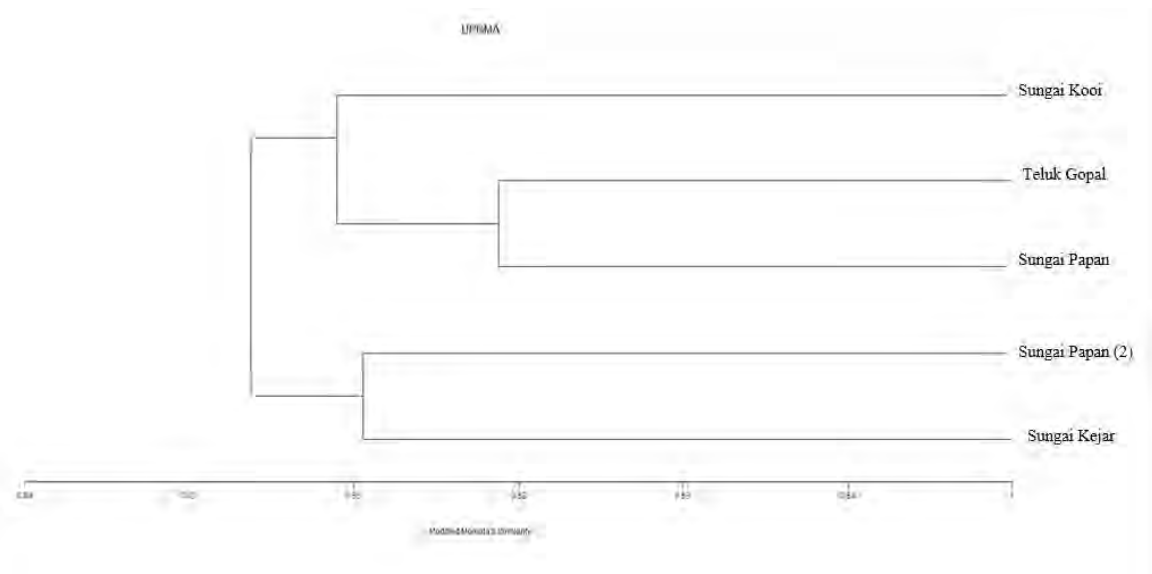
Figure 3. Tree diagram of clustering method (UPGMA) on liana community in the study sites of Royal Belum State Park.

The rarefied and extrapolated analysis of species richness showed an overlap in the confidence intervals of all the sites which indicated no significant difference between them (Figure 4). However, rarefied-extrapolated curves did not reach asymptote for all the sites, meaning that the sampling size was not adequate. The cluster analysis revealed the similarity of censuses trees species (Figure 5). The highest value was observed between Sungai Kooi and Teluk Gopal (0.27) followed by Sungai Papan and Sungai Papan 2 (0.23). Whilst, the comparison between Node 2 (consisting of Teluk Gopal and Sungai Kooi) and Node 1 (consisting of Sungai Papan and Sungai Papan 2) recorded the second highest at 0.20.

Notwithstanding, the lowest value was denoted between Sungai Kejar and Node 3 (consisting of four study sites) at 0.14, which suggests the lowest species similarity recorded between them.

IUCN Conservation Status of Lianas and Trees

The assessment of the IUCN status of the lianas showed six species as Least Concern while the others have Deficient Data (Table 4). However, the IUCN conservation status of the trees showed that two are Critically Endangered, three are Endangered, 11 are Vulnerable, eight are Near Threatened, 31 are Least Concern while the remaining 166 are Data Deficient (Table 5).

Table 3. Diversity indices of tree species in the study sites.

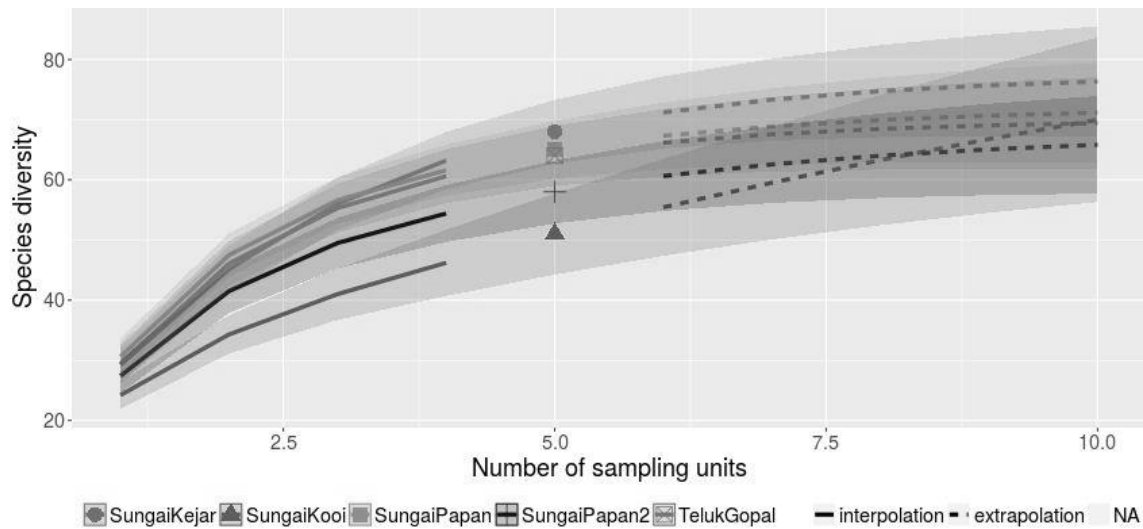| Diversity Indices	Sungai Kejar	Sungai Papan	Sungai Papan 2	Teluk Gopal	Sungai Kooi
Simpson Index	0.9809	0.9819	0.9788	0.9816	0.9730
Shannon Index	4.089	4.088	3.95	4.072	3.754
Evenness Index	0.865	0.904	0.880	0.903	0.821
Fisher alpha	49.13	42.59	37.86	42.99	33.14
Rarefied and extrapolated species richness	68	65	58	64	50

Figure 4. Incidence based rarefaction and extrapolation curves for species richness (including confidence intervals) of tree species. Solid lines represent rarefaction curves while dashed lines represent extrapolation curves. Each dot stands for the estimated species richness. The shadow of each curve represents the confidence interval.
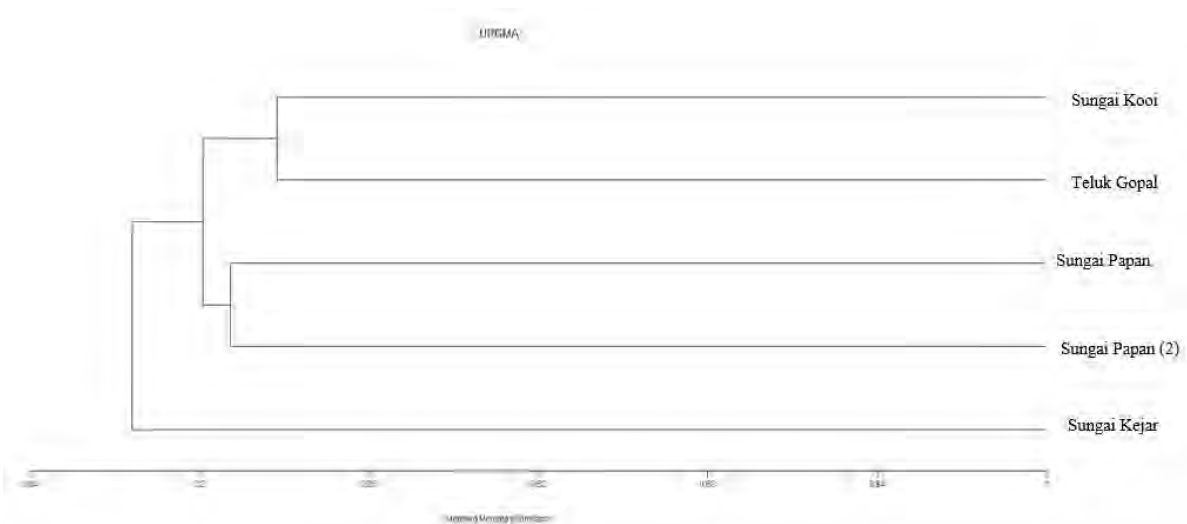

Figure 5. Tree diagram of clustering method (UPGMA) on liana community in the study sites of Royal Belum State Park.

Table 4. IUCN conservation status of lianas in the study sites.

IUCN Red List Status	Number of Species	Name of Species
Extinct (EX)	0	Nil
Extinct in the Wild (EW)	0	Nil
Regionally Extinct (RE)	0	Nil
Critically Endangered (CR)	0	Nil
Endangered (EN)	0	Nil
Vulnerable (VU)	0	Nil
Near Threatened (NT)	0	Nil
Least Concern (LC)	6	*Bauhinia acuminata, Spatholobus gyrocarpus, Gnetum gnemonoides, Gnetum latifolium, Gnetum macrostachyum, Gnetum microcarpum*
Data Deficient (DD)	86	Others

Table 5. IUCN conservation status of trees in the study sites.

IUCN Red List Status	Number of Species	Name of Species
Extinct (EX)	0	0
Extinct in the Wild (EW)	0	0
Regionally Extinct (RE)	0	0
Critically Endangered (CR)	2	*Dipterocarpus kunstleri, Shorea lepidota*
Endangered (EN)	3	*Parashorea densiflora, Shorea parvifolia, Shorea pauciflora*
Vulnerable (VU)	11	*Dipterocarpus gracilis, Hopea sangal, Hopea sublanceolata, Parashorea stellate, Vatica pauciflora, Beilschmiedia dictyoneura, Endocomia canarioides, Horsfieldia polyspherula, Horsfieldia sucosa, Pentace perakensis, Schoutenia kunstleri*
Near Threatened (NT)	8	*Shorea leprosula, Castanopsis curtisii, Aglaia leucophylla, Aglaia oligophylla, Aglaia palembanica, Aglaia rubiginosa, Aglaia squamulosa, Palaquium hexandrum*
Least Concern (LC)	31	*Anisophyllea corneri, Alphonsea maingayi, Endiandra maingayi, Tabernaemontana corymbosa, Canarium littorale, Canarium patentinervium, Dacryodes laxa, Dacryodes costata, Dacryodes puberula, Dacryodes rostrata, Dacryodes rugosa, Santiria apiculata, Santiria tomentosa, Santiria laevigata, Shorea multiflora, Diospyros ridleyi, Diospyros singaporensis, Paracroton pendulus, Ormosia macrodisca, Irvingia malayana, Beilschmiedia insignis, Chisocheton tomentosus, Sandoricum koetjape, Xylocarpus moluccensis, Knema conferta, Myristica iners, Prunus arborea, Prunus grisea, Payena maingayi, Celtis rigescens, Rinorea horneri*
Data Deficient (DD)	1	Others

DISCUSSION

The composition, abundance and diversity of lianas in some tropical forests has been directly influenced by the intensity of human disturbances (Schnitzer & Bongers, 2011; Addo-Fordjour & Rahmad, 2015). In these disturbed tropical forests, lianas became over-populated and exerted limiting effects on neighbouring plants, especially trees (Paul & Yavitt, 2011). This could mean that Sungai Papan and Teluk Gopak have undergone little human disturbances over the years. Favourable environmental conditions such as increased CO_2 level, sunlight availability and enough space for growth have been reported as promoters of liana diversity and abundance in disturbed tropical forests (Gerwing & Farias, 2000). The presence of tree species of

Annonaceae, Euphorbiaceae and Meliaceae could mean that these dominated plant families and genera are typical trees of tropical rain forests (Francoso *et al.,* 2016). The variations observed in the spread of these trees in all the sites in such a way that the abundant species in one site while less-abundant in the others and vice versa might just be as a result of delimitation in the geographical areas and time, not necessarily by physiological differences (Oliveira & Amaral, 2004).

This inventory and diversity assessments of trees in these study sites have been able to provide the understanding of the current richness status of the trees which could serve as a guide for driving the future conservation plans of these

forests (Jayakumar *et al.,* 2011; Francoso *et al.,* 2016). Researches have shown that the abundance of lianas in tropical forests usually reduced trees regeneration and interfered with ecosystem processes that tend to enhance trees richness and diversity (Garcia *et al.,* 2018). This means that abundance of lianas will directly reduce the abundance and diversity of trees in a forest community. Lianas are able to achieve this by competing with trees for water, soil nutrients and light (Perez-Salicrup *et al.,* 2001; Schnitzer *et al.,* 2005). The high tree species composition, richness and diversity recorded in our study revealed that these study areas have not been so much infested by liana species. The most important indicator of disturbances in a forest ecosystem is the high density of lianas compared to trees in such forests (Villagra *et al.,* 2013; Oliveira *et al.,* 2014). Invariably, most forests inside the Royal Belum State Park have not experienced a high level of disturbance over the years. According to the IUCN conservation status of the lianas and trees, it can be deduced that the most important tree species in this forest which are categorized as Endangered and Critically Endangered are *Parashorea densiflora, Shorea parvifolia, Shorea pauciflora Dipterocarpus kunstleri* and *Shorea lepidota.* None of the lianas can be described as important species for conservation. Also, lack of adequate data and assessment of the conservation status of most of these plants should be a serious concern to conservationists.

CONCLUSION

Royal Belum State Park encapsulates a mesmerizing diversity of lianas and trees together with the hospitable environmental condition. The similarity of species is considerably low suggesting high species richness. Both plant habits are important forest structure and are beneficial to animals, especially for protection. Therefore, it is reasonable to ensure the conservation of these forest resources. It is hereby suggested that the forest management should pay close attention to the most important tree species highlighted in this study, which stand the risk of going into extinction if not conserved immediately.

REFERENCES

Addo-Fordjour, P., Anning, A.K., Atakora, E.A. & Agyei, P.S. (2008). Diversity and distribution of climbing plants in a semi-deciduous rain forest, KNUST Botanic Garden, Ghana. *International Journal of Botany,* 4: 186-195.

Addo-Fordjour, P. & Rahmad, Z.B. (2015). Liana community assemblages in relation to human disturbance in a tropical forest in Ghana: implications for conservation. *International Journal of Biodiversity Science, Ecosystem Services & Management,* 10(4): 11-59.

Addo-Fordjour, P., Rahmad, Z.B. & Burham, R.J. (2016). Intercontinental comparison of liana community assemblages in tropical forests of Ghana and Malaysia. *Journal of Plant Ecology,* 10(6): 883-894.

Chao, A., Ma, K.H. & Hsieh, T.C. (2016). iNEXT (iNterpolation and EXTrapolation) Online: Software for Interpolation and Extrapolation of Species Diversity. Program and User's Guide. http://chao.stat.nthu.edu.tw/wordpress/software_download/. Downloaded on 7 May 2018.

Dixon, R.K., Brwon, R.A., Houghton, R.A., Solomon, A.M., Trexler, M.C. & Wisniewski, J. (1994). Carbon pools and flux of global forest ecosystems. *Science,* 263: 185-190.

Francoso, R.D., Haidar, R.F. & Machado, R.B. (2016). Tree species of South America central savanna: endemism, marginal areas and the relationship with other biomes. *Acta Botanica Brasilica,* 30(1): 78-86.

Garcia, L., Maria, M., Martinez, I., Laura, M., Felipe, N.A.P., Jennifer, S.S., Stefan, A. & Edwards, D. (2018). Lianas reduce community-level canopy tree reproduction in a Panamanian forest. *The Journal of Ecology,* 106(2): 737-746.

Gerwing, J.J. & Farias, D.L. (2000). Integrating liana abundance and forest stature into an estimate of total aboveground biomass for an eastern Amazonian forest. *Journal of Tropical Ecology,* 16: 327-335.

Ingwell, L.L., Wright, S.J., Becklund, K.K., Hubbell, S.P. & Schnitzer, S.A. (2010). The impact of lianas on 10 years of tree growth and mortality on Barro Colorado Island, Panama. *Journal of Ecology,* 98: 879-887.

Jayakumar, S., Kim, S.S. & Heo, J. (2011). Floristic inventory and diversity assessment - a critical review. *National Academy of Ecology and Environmental Sciences*, 1: 151-168.

Khairil, W.A., Suhani, I. & Hairani, M.N. (2012). Belum-Temengor Forest Complex: An eco-tourism perspective. *In* Hurzaid, A., Bakar, M.A., Sharma, D., Nasir, N., Sharma, R., Aznan, A.R., & Jaafar, I. (Eds.), *Proceedings of the 2nd Temengor Scientific Expedition*, August 22-25. Selangor: Pulau Banding Foundation. Pp 53-60.

Kiew, R., Chung, R.C.K., Saw, L.G., Soepadmo, E. & Boyce, P.C. (Eds.). (2010). *Flora of Peninsular Malaysia, Series II: Seed Plants*, *Volume 1*. Malaysia: Forest Research Institute Malaysia, Kepong.

Kiew, R., Chung, R.C.K., Saw, L.G., Soepadmo, E. & Boyce, P.C. (Eds.). (2011). *Flora of Peninsular Malaysia, Series II: Seed Plants, Volume 2*. Malaysia: Forest Research Institute Malaysia, Kepong.

Kiew, R., Chung, R.C.K., Saw, L.G. & Soepadmo, E. (Eds.). (2012). *Flora of Peninsular Malaysia, Series II: Seed Plants*, *Volume 3*. Malaysia: Forest Research Institute Malaysia, Kepong.

Krebs, C.J. (1989). *Ecological methodology*. New York: Harper & Row.

Ng, F.S.P. (1978). *Tree flora of Malaya: A manual for foresters, Volume 3*. Malaysia: Longman.

Ng, F.S.P. (1989). *Tree flora of Malaya: A manual for foresters*, *Volume 4*. Malaysia: Longman.

Oliveira, A.N. & Amaral, I.L. (2004). Florıstica efitossociologia de uma floresta de vertente na Amazonia Central, Amazonas, Brasil. *Acta Amazonica,* 34(1): 21-34.

Oliveira, E.A., Marimon, B.S., Feldpausch, T.R., Colli, G.R., Marimon-Junior, B.H., Lloyd, J., Lenza, E., Maracahipes, L., Oliveira-Santos, C. & Philips, O.L. (2014). Diversity, abundance and distribution of lianas of the Cerrado Amazonian forest transition. *Plant Ecology Diversity,* 7(1-2): 231-240.

Paul, G.S. & Yavitt, J.B. (2011). Tropical vine growth and the effects on forest succession: a review of the ecology and management of tropical climbing plants. *Botanical Review,* 77: 11-30.

Pérez-Salicrup, D.R. (2001). Effect of liana cutting on tree regeneration in a liana forest in Amazonian Bolivia. *Ecology,* 82: 389-396.

Perez-Salicrup, D.R., Sork, V.L. & Putz, F.E. (2001). Lianas and trees in a liana forest of Amazonian Bolivia. *Biotropica,* 33: 34-47.

Potter, C.S. (1999). Terrestrial biomass and the effects of deforestation on the global carbon cycle. *Bioscience,* 49: 769-778.

Putz, F.E. (2012). Vine ecology. *Ecology Information,* 24: 1-10.

Rahmad, Z.B. & Akomolafe, G.F. (2018). Distribution, diversity and abundance of ferns in a tropical university campus. *Pertanika Journal of Tropical Agricultural Science,* 41(4): 1875-1887.

Romesburg, H.C. (1984). *Cluster analysis for researchers*. Belmont, California: Lifetime Learning Publications.

Schnitzer, S.A. & Bongers, F. (2011). Increasing liana abundance and biomass in tropical forests: emerging patterns and putative mechanisms. *Ecology Letter,* 14: 397-406.

Schnitzer, S.A., Dalling, J.W. & Carson, W.P. (2000). The impact of lianas on tree regeneration in tropical forest canopy gaps: evidence for an alternative pathway of gap-phase regeneration. *Journal of Ecology,* 88: 655-666.

Schnitzer, S.A., Kuzee, M.E. & Bongers, F. (2005). Disentangling above and below-ground competition between lianas and trees in a tropical forest. *Journal of Ecology,* 93: 1115-1125.

Tobin, I., Bony, S. & Roca, R. (2012). Observational evidence for relationships between the degree of aggregation of deep convection, water vapor, surface fluxes, and radiation. *Journal of Climate*, 25: 6885-6904.

Villagra, B.L.P., Gomes, E.P.C., Burnham, R.J. & Neto, S.R. (2013). Diversity and abundance of climbers from the Atlantic Forest, Southeastern Brazil. *Biodiversity and Conservation,* 22(11): 2505-2517.

Whitmore, T.C. (1983a). *Tree flora of Malaya: a manual for foresters*, *Volume 1*. Malaysia: Longman.

Whitmore, T.C. (1983b). *Tree flora of Malaya: a manual for foresters*, *Volume 2*. Malaysia: Longman.

Population Estimation of Proboscis Monkeys in Mangroves at Kuching Wetland National Park, Sarawak

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ABSTRACT

Boat survey on proboscis monkey (*Nasalis larvatus*) population in Kuching Wetland National Park (KWNP) was conducted to estimate the current population density and population size of this primate. The survey was conducted on September 2015 and January 2016 covering a cumulative distance of 128.91 km of mangrove riverbank. A cumulative total of 158 individuals comprising 19 groups, including one all-male group and three solitary males were recorded throughout the survey. The population density of proboscis monkeys in mangrove forest at KWNP was estimated at 1.63 individuals/km^2 or 0.20 groups/km^2. Based on the extrapolation of the estimated population density data, the population size of proboscis monkey in mangrove forest at KWNP was estimated to be 82 individuals. Last published report on the estimation of proboscis monkey population in Sarawak was more than 30 years ago. This study was conducted as a part of the efforts to assess the current population status of proboscis monkey in Sarawak.

Keywords: Estimation, population density, population size, proboscis monkey

INTRODUCTION

Proboscis monkey (*Nasalis larvatus*) is a large sexually dimorphic Colobine primate that is endemic to Borneo. It occurs in Brunei, Indonesia (Kalimantan) and East Malaysia (Sabah and Sarawak). An adult male proboscis monkey can be easily identified by its large and pendulous nose. However, an adult female proboscis monkey possesses a smaller and pointed nose (Phillipps & Phillipps, 2016). In both sexes, proboscis monkey has reddish-brown fur but a male has more striking and contrasts in colour, with a mane hair behind its back (Bennett & Gombek, 1993). The weight of an adult male proboscis monkey can reach up to 24 kg, while an adult female is usually half of this size, making the proboscis monkey to be the largest Colobine monkey (Allen & Coolidge, 1940; Schultz, 1942; Wolfheim, 1983; Phillipps & Phillipps, 2016). An infant proboscis monkey is born with dark brown fur and its face is covered with dark blue colour (Bennett & Sebastian, 1988).

The natural habitats of proboscis monkey are restricted to the lowland coastal rainforests and always associated with waterways including mangroves, riverine, peat and fresh water swamp forests (Kawabe & Mano, 1972; Salter *et al*., 1985; Bennett & Gombek, 1993; Meijaard & Nijman, 2000). During the day, it will forage inland, normally less than 1 km away from the riverbank and always return before dusk to sleep (Salter *et al*., 1985; Bennett & Sebastian, 1988; Yeager, 1989; Matsuda *et al*., 2008). The basic social unit of proboscis monkey is a harem group that consists of an adult male, several females and their offsprings (Bennett & Sebastian, 1988). Within several groups, a secondary level of social organisation may occur where they may travel and sleep together in close proximity (Bennett & Sebastian, 1988).

In the Red List of Threatened Species, International Union for the Conservation of Nature (IUCN), proboscis monkey is classified as an Endangered species (IUCN, 2017) and is listed in Appendix I, in the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES). Similar to other primates, habitat degradation and destruction become the major threats to the survival of proboscis monkey in Borneo (Meijaard & Nijman, 2000). The population trends of proboscis monkey showed a great decreased by more than 50% during the past 40 years (Meijaard *et al*., 2008). In Sarawak, many of the

natural habitats of proboscis monkey are exposed to logging, agriculture and tourism activities (Salter & MacKenzie, 1985). The degradation of mangroves and peat swamp forests generally leads to a reduction of the proboscis monkey population (Bennett, 1988).

Mangrove forest is one of the preferred habitats for proboscis monkey (Phillipps & Phillipps, 2016). Mangrove forest not only provides refuge for proboscis monkey but it is also economically beneficial. In KWNP alone, mangrove forest supported fisheries worth for more than US$21.1 million. In addition, mangrove forest in KWNP yielded annually US$123,217 in timber production, US$3.7 million from the tourism industry and provided for more than 3,000 jobs for the locals (Bennett & Reynolds, 1993). KWNP is the only area in Sarawak that remains as an important refuge for mangrove flora and fauna, including proboscis monkey (Bennett & Reynolds, 1993).

Other than KWNP, there are several localities in Sarawak where the proboscis monkeys can be found including Bako National Park, Samunsam Wildlife Sanctuary, Maludam National Park, Limbang Mangrove National Park and Kuala Lawas Forest Reserve. However, to date, there are still lack of efforts to update the population status of this endangered species. The status of the proboscis monkey population in Sarawak is still depending on the outdated data done in the 80s (Salter & MacKenzie, 1985; Bennett et al., 1987). Thus, this study was conducted to estimate the current population status of proboscis monkey in mangrove forest at KWNP, and to update the current estimation of proboscis monkeys left in Sarawak. It is important to have a reliable and updated data on the population size because this is the basic information needed when constructing a conservation plan.

MATERIALS AND METHODS

Study Area

The survey was conducted at KWNP, located approximately 15 km from Kuching City, Sarawak. Previously, this park was known as Sarawak Mangrove Forest Reserve before it was gazetted as a national park in 2000, with an area of 6,610 ha. On 8th November 2005, KWNP was designated as the first Ramsar Site in Sarawak under the Ramsar Convention (Beavitt & Tuen, 2010). Surveys were concentrated in the mangrove forest at KWNP as mangroves cover almost 70% of the total area of the park (Table 1).

Survey Technique

Boat survey on proboscis monkey in KWNP was conducted following Bennett (1986). This survey was conducted by direct observation along the mangrove riverbank in the early morning and late evening. In the morning sessions, the surveys were started at dawn and finished about 90 minutes later. While in the evening sessions, the surveys were started about 60-90 minutes before dusk. Surveys were conducted only in these limited periods of time as to adapt to the natural behaviour of the proboscis monkeys that sleep at the riverbank (Payne & Francis, 2007). In order to cover the large mangrove area in KWNP, the surveys were designated into two parts. During the first part of the survey, which was conducted on 30th September until 2nd October 2015, the survey covered the western part of the park, mainly in Sungai Sibu Laut. The second part of the survey was conducted on 11th January until 13th January 2016. At this time, surveys covered the eastern part of the park, specifically along Batang Salak (Figure 1). In each part of the survey, three days were allocated allowing at least six trips of surveys in each part. The one-way survey was conducted to prevent double

Table 1. Total land use areas based on geospatial analysis at Kuching Wetland National Park.

Vegetation Type	Total Area (km^2)	Proportion (%)
Bare Land	0.60	0.82
Cultivated Land/ Secondary Vegetation	1.61	2.20
Infrastructure/ HQ	0.37	0.50
Tropical Heath	7.47	10.23
Mangrove	49.97	68.40
Peat Swamp	3.04	4.17
Water body	10.00	13.68
Total	73.06	100.00

Figure 1. Survey route in KWNP (Source: Google Earth Pro 7.1.8.3036).

counting of individual of proboscis monkey. At least two enumerators were involved during the survey.

Data Collection

Once any proboscis monkey individuals were sighted, the boat engine was immediately switched off, to avoid further disturbance and causing the proboscis monkey to move away from the location. The size of the group was recorded and identified into sex and age classes based on Bennett and Sebastian (1988). The coordinates of each group sighted were recorded by using Geographic Positioning System (Garmin 64s). Any individuals of proboscis monkey within 50 m radius were considered as the same group.

Mapping and Area Measurement Analysis

The soil map of Sarawak dated in 1968 (source from Department of Agriculture, Soil Survey Division) was updated and rectified to estimate the current mangrove area in KWNP (Figure 2). This process was done by using Geographic Information System software programme (QGIS version 2.14.10) with the aids of current forest cover, obtained from the satellite image of Landsat 8, Sentinel 2 MODIS 2016 and SRTM 1-arcsecond. Soil map was used in the rectification process since soil type is strongly correlated with forest type distribution (Hazebroek & Abang Kashim, 2006). Additional, land use maps were acquired from the Department of Survey and Mapping Sarawak, including map of the global distribution of mangroves created by UNEP-WCMC-Global in 2011 and Sarawak topography map series DNMM5201.

Data Analysis

The population density of proboscis monkeys in KWNP was estimated by dividing the cumulative number of individuals sighted with the total surveyed area. The total surveyed area was the cumulative distance of the surveyed riverbank multiplied with 0.75 km perpendicular distance from the riverbank. The analysis of the estimated population size was correlated with the population density obtained. The estimated population density of proboscis monkey was multiplied by the total area of mangrove forest in KWNP to obtain the estimated population size of proboscis monkey in the mangrove forest at KWNP. The formulas used were as below:

Population density =
Cumulative number of individuals sighted
Total surveyed area (sq km)

Total surveyed area = Cumulative distance of surveyed riverbank (km) x *0.75 km*

Population size = Population density x *total area of mangrove forest in KWNP*

Home range size plays an important role in the estimation of population density of proboscis monkey (Bernard & Hamzah, 2006). In order to obtain the home range size of proboscis monkey in KWNP, a periodic observation is needed which could not be conducted in this study. Thus, perpendicular distance of 0.75 km from the riverbank was applied in this study to estimate the total surveyed area by multiplying the cumulative distance of surveyed riverbank with 0.75 km. This figure was adopted from the study
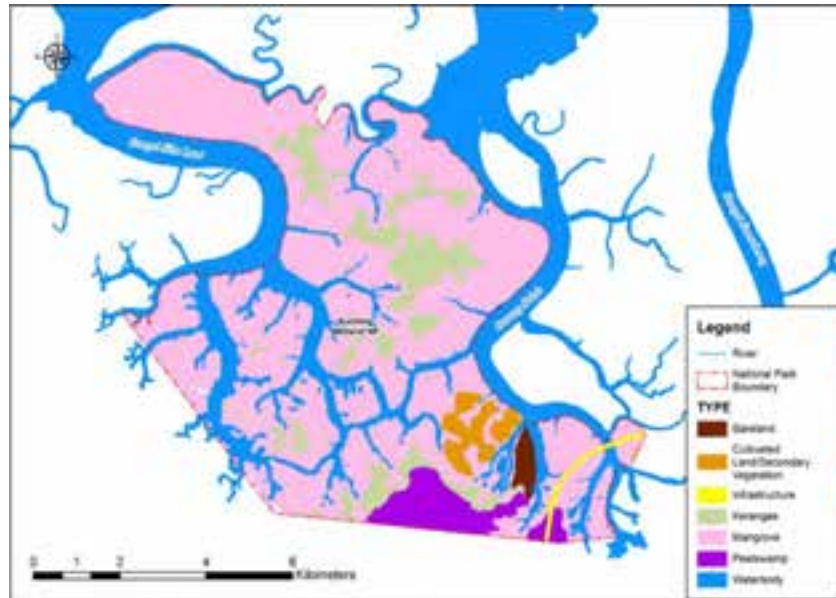
Figure 2. Vegetation map of KWNP (Source: Department of Agriculture, Soil Survey Division).

on the ranging behaviour of proboscis monkey in Samunsam Wildlife Sanctuary and Bako National Park. The distance of 0.75 km is the maximum perpendicular distance from the riverbank where the proboscis monkey might travel from its sleeping sites (Salter *et al*., 1985). This distance is applied in this study, since this is the closest and available ranging distance that was recorded in Sarawak. Besides, it is still in the range of the daily movement of proboscis monkey that have been reported (Bennett & Sebastian, 1988; Boonratana, 2000; Sebastian, 2000; Bismark, 2010).

RESULTS

A cumulative distance of 128.91 km of mangrove riverbank was surveyed over the six-day survey in this park. A cumulative of 158 individuals from 15 harem groups, one all-male group and three solitary males of proboscis monkeys were sighted throughout the survey. Based on the analysis, the population size of the proboscis monkey in KWNP was estimated at 82 individuals with the estimated population density of 1.63 individuals/km^2 or 0.20 groups/km^2 (Table 2).

DISCUSSION

No studies on estimation of proboscis monkey based on vegetation have been conducted previously. The analysis of this study was designed to estimate the population density and population size based on the vegetation types where the proboscis monkeys were encountered. The estimated population density of proboscis monkey was extrapolated only to the mangrove area to estimate the population size. Thus, the extrapolation of the population density was focused on mangrove forest rather than extrapolating the population density to the whole area of KWNP, which could result in over estimation. In addition, the estimated population density itself has represented the population of proboscis monkey in mangroves but not for the whole area of the KWNP.

The usage of a cumulative number of individuals in the analysis of population density was found to be more precise than other previous studies, which used the maximum number of individuals in their analysis (Bernard & Hamzah, 2006; Ali *et al*., 2009). The cumulative number applied in the analysis of the population density of this study was actually representing the

Table 2. The estimated population of proboscis monkey in mangroves at KWNP.

Cumulative number of individuals sighted	Cumulative number of groups sighted	Cumulative distance of surveyed riverbank (km)	Total surveyed area (km^2)	Total forest area (km^2)	Estimated population density		Estimated population size
					Individuals/km^2	Groups/km^2	
158	19	128.91	97.68	49.97	1.63	0.20	82

average individuals, as it was divided by the cumulative surveyed area. In order to cover as much area of a survey within the limited period of the survey in every session, the use of maximum number could be less applicable. However, the usage of maximum number might be applicable only if the distance of the survey were fixed. Moreover, it has to be implemented within the limited period of survey for every session and the survey covered the same river for every trip.

The number of proboscis monkey in KWNP was found to be unusually different between both parts of the surveys. The number of sighted groups during the first part of the survey was almost six times lower than the second part. The population was found to be more around Batang Salak with 16 groups recorded while only three groups were recorded along Sungai Sibu Laut. This situation of less proboscis monkey along Sungai Sibu Laut was probably due to the heavy usage of the river by the local communities as it is near to Telaga Air town, the main town for the surrounding local people. Some groups of the proboscis monkey were sighted outside of the national park boundary, the other side of the river. These groups were also taken into account since proboscis monkey is known for its ability to swim across the river (Bennett & Sebastian, 1988). Proboscis monkey has been reported to swim underwater for up to 20 m to avoid predation (Bennett & Gombek, 1993).

Based on the observation during the survey, there were disturbances toward the proboscis monkey population in KWNP that may be worth to be mentioned. Firstly, the use of the area by locals for their economic activities, which are wood pole collection and fishing activities. These activities were not seemed to affect the proboscis monkey adversely but it limited the habitat used by the proboscis monkeys as they were avoiding those areas with human-induced disturbances (Salter *et al*., 1985). Removal of poles, especially from *Rhizophora* sp., does not break the canopy of the mangrove. Pole collection was selective and like the fishing activities, it was conducted on a small scale by the local community. However, cautious also need to be taken given this park is easily accessible by the public for fishing activities. Poachers from outside of the area may pretend that they come for fishing, but actually they may hunt for proboscis monkeys. The natural behaviour of this species that spends most of its time at the riverbank, makes these animals more vulnerable to be hunted (Bennett, 1987). Routine patrolling is needed in this park to avoid illegal hunting activities.

An ongoing mining activity in Pulau Salak is a more severe disturbance to the population of proboscis monkey in KWNP. Even though Pulau Salak is not within the boundary of the park, a big number of proboscis monkeys were recorded from this area. A huge area of Pulau Salak was cleared for mining a limestone quarry which directly affects the habitat of proboscis monkey. Within Pulau Salak, there is a small Malay village known as Kampung Salak. Hunting activities by local communities, especially from Kampung Salak and nearby villages, were not in existence based on the informal interview and personal observations did during the survey. This is probably because the majority of the nearby villagers are Muslims and it is taboo for them to consume this animal.

CONCLUSION
The population size of proboscis monkey in the mangrove vegetation at KWNP was estimated at 82 individuals with the estimated population density of 1.63 individuals/km^2 or 0.20 groups/km^2. Survey on proboscis monkey population in Sarawak should be a continuous endeavour. Updated and reliable data on the population estimation of proboscis monkey is urgently needed in the efforts to construct a relevant conservation plan to ensure the survival of this animal. This is also true for other important proboscis monkey sites, to obtain an estimation of population size and population density for the whole area of Sarawak.

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REFERENCES

Ali, M.R., Bernard, H. & Hanya, G. (2009). The population size and distribution of proboscis monkeys (*Nasalis larvatus*) based on a brief study in Garama, Klias Peninsula, Sabah, Malaysia. *Journal of Tropical Biology and Conservation*, 5: 67-70.

Allen, G.M. & Coolidge, H.J. (1940). Asiatic primate expedition collections: mammals. *Bulletin of the Museum of Comparative Zoology*, 87: 131-166.

Beavitt, W. & Tuen, A.A. (2010). *Kuching Wetlands National Park (SWAMP): a photographic guide to the plants and animals of Malaysia's coastal and mangrove habitats*. Kota Samarahan: Universiti Malaysia Sarawak Publication. P 3.

Bennett, E.L. & Gombek, F. (1993). *Proboscis monkeys of Borneo*. Natural History Publications (Borneo) Sdn. Bhd. & Koktas Sabah, Ranau, Sabah, Malaysia. Pp 84-99.

Bennett, E.L. & Reynolds, C.J. (1993). The value of a mangrove area in Sarawak. *Biodiversity and Conservation*, 2(4): 359-375.

Bennett, E.L. & Sebastian, A.C. (1988). Social organization and ecology of proboscis monkeys (*Nasalis larvatus*) in mixed coastal forest in Sarawak. *International Journal of Primatology*, 9(3): 233-255.

Bennett, E.L., Caldecott, J.O., Kavanagh, M. & Sebastian, A.C. (1987). Current status of primates in Sarawak. *Primate Conservation: The Newsletter and Journal of the IUCN/SSC Primate Specialist Group*, 8: 184-186.

Bennett, E.L. (1986). *Proboscis monkeys in Sarawak: their ecology, status, conservation and management*. World Wildlife Fund Malaysia, Kuala Lumpur and New York Zoological Society, New York. Pp 94-99.

Bennett, E.L. (1987). The value of mangroves in Sarawak. *Sarawak Gazette*, 63: 12-21.

Bennett, E.L. (1988). Proboscis monkeys and their swamp forests in Sarawak. *Oryx*, 22(2): 69-74.

Bernard, H. & Hamzah, Z. (2006). Population size and distribution of the proboscis monkey (*Nasalis larvatus*) in the Klias Peninsula, Sabah, Malaysia. *Malayan Nature Journal*, 56(2): 153-163.

Bismark M. (2010) Proboscis monkey (*Nasalis larvatus*): bio-ecology and conservation. *In* Gursky-Doyen, S. & Supriatna, J. (Eds.), *Indonesian primates: developments in primatology: progress and prospects*. New York: Springer. Pp. 217-233.

Boonratana, R. (2000). Ranging behavior of proboscis monkeys (*Nasalis larvatus*) in the Lower Kinabatangan, northern Borneo. *International Journal of Primatology*, 21(3): 497-518.

Hazebroek, H.P. & Abang Kashim, A.M. (2006). *National Parks of Sarawak*. Kota Kinabalu: Natural History Publications (Borneo). Pp 313-345.

IUCN. (2017). International Union for Conservation of Nature (IUCN) Red List of Threatened Species Version 2017.2. https://www.iucnredlist.org./ Downloaded on 26 May 2017.

Kawabe, M. & Mano, T. (1972). Ecology and behaviour of the wild proboscis monkey, *Nasalis larvatus* (Wurmb), in Sabah, Malaysia. *Primates*, 13(2): 213-227.

Matsuda, I., Tuuga, A., Akiyama, Y. & Higashi, S. (2008). Selection of river crossing location and sleeping site by proboscis monkeys (*Nasalis larvatus*) in Sabah, Malaysia. *American Journal of Primatology*, 70(11): 1097-1101.

Meijaard, E. & Nijman, V. (2000). Distribution and conservation of the proboscis monkey (*Nasalis larvatus*) in Kalimantan, Indonesia. *Biological Conservation*, 92(1): 15-24.

Meijaard, E., Nijman, V. & Supriatna, J. (2008). *Nasalis larvatus* The IUCN Red List of Threatened Species 2008. https://www.iucnredlist.org/details/14352/0. Downloaded on 25 January 2018.

Payne, J. & Francis, C.M. (2007). *A field guide to the mammals of Borneo*. Kota Kinabalu: The Sabah Society. Pp 228.

Phillipps, Q. & Phillipps, K. (2016). *Phillipps' field guide to the mammals of Borneo and their ecology: Sabah, Sarawak, Brunei, and Kalimantan*. USA: Princeton University Press. Pp. 174-179.

Salter, R.E. & MacKenzie, N.A. (1985). Conservation status of proboscis monkey in Sarawak. *Biological Conservation*, 33(2): 119-132.

Salter, R.E., MacKenzie, N.A., Nightingale, N., Aken, K.M. & Chai, P.K.P. (1985). Habitat use, ranging behaviour and food habits of the proboscis monkey, *Nasalis larvatus* (van Wurmb), in Sarawak. *Primates*, 26(4): 436-451.

Schultz, A.H. (1942). Growth and development of the proboscis monkey. *Bulletin of the Museum of Comparative Zoology at Harvard College*, 89(6): 279-314.

Sebastian, A.C. (2000). Proboscis monkey in Danau Sentarum National Park. *Borneo Research Bulletin,* 31: 359-371.

Wolfheim, J.H. (1983). *Primates of the world: distribution, abundance and conservation*. Seattle: University of Washington Press. Pp 553.

Yeager, C.P. (1989). Feeding ecology of the proboscis monkey (*Nasalis larvatus*). *International Journal of Primatology,* 10(6): 497-530.

Effect of *Trichoderma* sp. on Anthracnose Disease of Stored Chilli

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ABSTRACT

Chilli is commonly used as spice in Malaysian culinary, principal ingredients in paste (*sambal*) and as the raw material in sauce industry. Anthracnose disease caused by *Colletotrichum capsici* is one of the major causes of economic loss to chilli production especially in Asia. Even a small lesion on chilli might affect the quality, thus the market value of the chilli. Disease symptoms caused by *C. capsici* include brown, circular and sunken lesion with concentric rings of black acervuli. Chemicals have been used to treat the chilli but they might cause environmental pollution, affect human health and lead to pathogen resistance to the chemicals. Therefore, an alternative method to chemical control is required. In this study, *C. capsici* was isolated from a naturally infected chilli fruit (*Capsicum frutescens*), and a species of *Trichoderma* was isolated from the rhizosphere of grasses. Pure cultures of both fungi were established then used in antagonism studies in *in vitro* and *in vivo*. Dual culture of pathogens and *Trichoderma* sp. indicated that *Trichoderma* sp. competed with *C. capsici* for space and nutrients, caused the loss of turgidity of the fungal hyphae, and reduced the fungal growth by producing volatile metabolites. *Trichoderma* sp. decreased disease severity on chilli artificially inoculated fruits up to 64% when *Trichoderma* mycelial plug was used and 55% when culture filtrate was applied. Field trials are recommended to examine the antagonism of *Trichoderma* sp. in real production conditions.

Keywords: Anthracnose, biological control, *Colletotrichum capsici*, *Trichoderma* sp.

INTRODUCTION

Capsicum frutescens L. or commonly known as bird eye chilli (Williams *et al*., 1991) belongs to the family Solanaceae and in the Plantae kingdom. Chilli is one of the most important spice crops in the world (Rahman *et al*., 2013) and in Malaysia it is used as one of the principal ingredients in paste (*sambal*) (Karim *et al*., 2011). Yet, chilli has been attacked by many diseases, and among them, fungal disease is the most important (Rahman *et al*., 2013).

Anthracnose or commonly known as ripe fruit rot is one of the major causes of economic loss to chilli production (Than *et al*., 2008a; Than *et al*., 2008b), especially in Asia (Sangdee *et al*., 2011). According to Pandey and Pandey (2003), anthracnose caused yield loss of more than 50% in chilli production in India. Initially, small and circular water-soaked spots will first develop on the skin (Naipagropediaraichur, 2012). The infected surface of the fruit will then sunken and dry up (Than *et al*., 2008a). Anthracnose is caused by *Colletotrichum* spp. (Than *et al*., 2008b). Kim *et al*. (2014) reported at least five

species, which are *C. gloeosporioides, C. acutatum, C. coccoides, C. dematium* and *C. truncatum* were associated with the anthracnose in chilli.

Chemicals have been used to control the anthracnose of chilli (Benítez *et al*., 2004). However, resistance to the chemicals has been reported for the pathogen of anthracnose (Benítez *et al*., 2004). In addition, the extensive use of the chemicals might lead to the pollution of the environment and the health of both growers and consumers. In order to reduce the usage of chemicals on the control of chilli anthracnose, alternative control approaches are needed (Rahman *et al*., 2011).

Antagonists, also known as biological control agents, are mostly soil microorganisms that can interfere with pest's activities (Chernin & Chet, 2002). There are four mechanisms of antagonists, which are competition, antibiosis, induced resistance and parasitism. *Trichoderma* spp. are one of the popular fungi known for their antagonism against soil pathogen such as *C. truncatum* which causes anthracnose on chilli

plants (Chernin & Chet. 2002; Verma *et al*., 2007). They are being used widely as biological control agents in many countries (Rahman *et al*., 2011). Hill *et al*. (2010) reported that selected *Trichoderma* isolates helped to enhance the health of *Acacia mangium* seedlings in Sarawak, Malaysia. Another study by Padder and Sharma (2011) proved that *Trichoderma viride* had the best potential to inhibit mycelial growth and spore germination of *C. lindemuthianum*.

The objectives of this study were: (1) to study the effect of *Trichoderma* sp. on the growth of anthracnose pathogen of chilli on culture medium, and (2) to investigate the efficacy of *Trichoderma* sp. in suppressing development of anthracnose disease on artificially inoculated chilli fruits.

MATERIALS AND METHODS

Infected fruits of *Capsicum frutescens* L. with characteristic symptoms of anthracnose for pathogen isolation and healthy fruits for artificial inoculation were purchased locally.

Pathogen Isolation and Pure Culture Establishment

Naturally infected *Capsicum frutescens* L. fruits showed the characteristic symptoms of anthracnose including sunken lesion on the fruit skin, which contained numerous black acervuli of conidia masses on the surface of the lesion were used for the isolation (Figure 1). The conidia, when observed under a compound

Figure 1. Fruits of bird eye chillies with characteristic symptoms of anthracnose.

microscope at 400× magnification, appeared to be tapering towards each end with acute apex and truncate base.

Potato Dextrose Agar (PDA) was used for the isolation of associated pathogen, which followed the method described by Ratanacherdchai *et al*. (2010). The infected fruits were first surface sterilised with 5% of Clorox® bleach (10% sodium hypochlorite) for two minutes and rinsed three times with sterile distilled water. Three sterilised specimens excised from the borderline of infected and healthy area were then placed onto each of five PDA replicate plates, and the plates were incubated for four to seven days at room temperature (approximately 28 °C). Pure culture of the pathogen was obtained using single spore isolation method (Choi *et al*., 1999). Briefly, a loopful of spore mass of 7-day old culture was transferred into a centrifuge tube which contained 10 ml of sterile distilled water, and the solution was gently shaken to disperse the spore mass. The solution was then poured onto PDA plates and left to stand for 5 minutes. The excessive solution on the plates was then poured off, and the plates were incubated slanting for 12 to 18 hours. Single spores of *Colletotrichum capsici* that germinated between 18 to 24 hours were transferred onto new PDA plates. The isolated *C. capsici* was identified based on the descriptions by Liu *et al*. (2016), based on the macro- and microscopic structures of the fungus.

Pathogenicity Test

Before surface sterilization, healthy fruits of *Capsicum frutescens* L. were washed with running tap water for half an hour to remove any contaminants on the surface of the fruits. The fruits were then surface sterilised with 70% ethanol for five minutes based on the method described by Sangdee *et al*. (2011). The sterilised fruits were then washed with sterile distilled water and left to dry on a sterilised filter paper in a laminar flow. Two wounds per fruit were created on sterilised chilli fruits using sterilised scalpel, and a plug of a 7-day old pure culture of the fungus was transferred and inserted into the wounds. Chilli fruits which served as controls had PDA plugs inserted into the wounds. The inoculated fruits were then

placed in transparent plastic boxes. There were five replicates boxes (five fruits per box) for each treatment and the boxes were kept at room temperature and observed daily. Symptoms at the inoculation site were evaluated one week after the inoculation (Montri *et al.*, 2009) and compared with the symptoms observed from the naturally infected fruits. Re-isolation of the fungus from the artificially inoculated fruits was carried out for comparison with the fungus isolated from the naturally infected fruits. The infected tissues were taken from about 1cm from the point of inoculation and underwent surface sterilization before being transferred onto new PDA plates. The plates were then incubated at room temperature, and the colonies were observed for the spore and colony characteristics.

Isolation of Antagonistic Fungus

Trichoderma sp. was isolated from rhizosphere soil of grasses following the method described by Bharathi *et al.* (2004). Rhizosphere soil samples were collected and diluted in test tubes up to 10^{-6}. Pure culture of *Trichoderma* sp. was established using single spore isolation (Choi *et al.*, 1999), as described earlier for the establishment of *Colletotrichum* pure culture. Identification of the isolated *Trichoderma* sp. was based on the description by Shah *et al.* (2012) by observing the macro- and microscopic structures of the fungus. The pure culture of *Trichoderma* sp. was subsequently used in antagonism test in culture plate with *Colletotrichum* sp. and on artificially inoculated chilli fruits.

Antagonistic Activity of the *Trichoderma* sp. on Culture Plate

Isolated *Trichoderma* sp. was screened for its antagonistic activity against *Colletotrichum* sp. using dual culture technique described by Begum *et al.* (2008). Briefly, an agar disc containing mycelia of 7-day-old *Trichoderma* sp. culture was placed at one end of the agar plate while an agar disc containing mycelia of 7-day-old *Colletotrichum* sp. culture was placed at the other end on the same PDA plate (hereinafter referred to as Treated plate). Plates, which served as controls, had agar disc of *Colletotrichum* sp. mycelia and a disc of PDA placing opposite each other on PDA plates.

There were five replicate plates for each treatment, and the plates were incubated for 15 days at room temperature. The plates were observed daily, and the average growth rate of *Colletotrichum* sp. and *Trichoderma* sp. was computed using the formula described by Rosli (2017) (Eq. 1) and the antagonistic activity of the *Trichoderma* sp. toward *Colletotrichum* sp., expressed as percentage of inhibition of radial growth was determined using the formula described by Begum *et al.* (2008) (Eq. 2) as below.

Average growth rate:

$$\frac{(D2-D1)+(D3-D2)+(D4-D3)+[DN-D(N-1)]}{N-1} \quad \text{Eq. (1)}$$

Where D indicates the average colony radial of *Colletotrichum* sp. and N indicates the number of days after incubation.

Percent inhibition of radial growth:

$$\frac{R1-R2}{R1} \times 100 \quad \text{Eq. (2)}$$

Where R1 indicates the radial growth of the fungal colony of the control set, and R2 indicates the radial growth of the fungal colony of the treated set.

Scanning Electron Microscopy (SEM) was used to observe the interaction between the two fungi after the *Trichoderma* sp. had grown over the *Colletotrichum* sp. on the dual culture plates. Agar plugs from confrontation zone were fixed in phosphate buffer solution (pH 7) for 24 hours as described by Carvalho *et al.* (2014). Phosphate buffer solution was prepared by combining the stock solution of 1 M of dipotassium phosphate (K_2HPO_4) and 1 M of monopotassium phosphate (KH_2PO_4) to 1 l of distilled water. After 24 hours, the samples were rinsed with fresh buffer solution for three times, and the solution was replaced by the lowest concentration of ethanol solution and left for one hour for dehydration purpose. The ethanol concentrations used were 60%, 80% and 100%. Lastly, the samples were placed in 100% ethanol overnight. The samples were then dried with carbon dioxide in a critical point dryer, mounted on aluminium stubs with double-sided tape and coated by gold. The samples were visualized by using a SEM (JEOL, JSM-639OLA).

Action of Volatile Metabolites of *Trichoderma* sp. on *Colletotrichum* sp.

For volatile metabolites test, technique described by Muthukumar *et al*. (2011) was used with modification by sealing the plates together with parafilm. Briefly, agar disc containing mycelia of 7-day-old *Trichoderma* sp. culture and *Colletotrichum* sp. culture were placed separately at the centre of the bottom of each PDA plate, respectively. Next, the lids of the PDA plates which contained *Trichoderma* sp. agar disc were replaced by the bottom of PDA plates which contained *Colletotrichum* sp. agar disk. The two plates were sealed together with parafilm (hereinafter referred to as Treated plate). Plates served as the controls were prepared in the same manner, except that PDA discs were used instead of *Trichoderma* sp. mycelial discs (hereinafter referred to as Control plate). There were five replicate plates for each treatment, and the plates were incubated at room temperature until the mycelia of *Trichoderma* sp. was observed to start growing over the other plate containing *Colletotrichum* sp.. The plates were observed daily, and percentage of inhibition of radial growth of the pathogen was determined. Formula described by Rosli (2017) was used to record the growth of *Colletotrichum* sp. and *Trichoderma* sp.. Formula described by Begum *et al*. (2008) was used to analyse the antagonistic activity of *Trichoderma* sp.

Inhibitory Efficacy of *Trichoderma* sp. against Anthracnose Pathogen Growth and Disease Development on Chilli Fruits

Healthy fruits were first inoculated artificially with *Colletotrichum* sp. then treated with *Trichoderma* sp. based on method described by Kim *et al*. (2014) with modification by creating the wounds using sterilised scalpel. Briefly, healthy fruits were surface sterilised with 70% ethanol for five minutes. The sterilised fruits were then washed with sterile distilled water and left to air dry on a sterilised filter paper in a laminar flow. A plug of 7-day old *Colletotrichum* sp. culture was transferred and inserted into a sterilised fruit after the wound was created on the surface of the fruit using a sterilised scalpel. A second wound was made approximately 1 cm apart from the initial wound, and a plug of 7-day old *Trichoderma* sp. culture was transferred and inserted into the wound. Chilli fruits, which had *Colletotrichum* sp. culture and a PDA plug inserted into the first and second wound, respectively, served as the controls. Treated chilli fruits were placed separately in plastic containers, three chilli fruits per container, for each treatment, and the containers were arranged in a Completely Randomised Design. There were five replicate containers for each treatment, and the containers were incubated for 15 days at room temperature. Disease incidence and severity were recorded and computed using Eq (3) and Eq. (4).

Percent of fruit rot incidence =

$$\frac{\text{No. of fruit rot}}{\text{Total no. of fruit}} \times 100 \qquad \text{Eq. (3)}$$

(Ngullie *et al*., 2010)

Percent of disease severity =

$$\frac{(R - C)}{R} \times 100 \ \times 100 \qquad \text{Eq. (4)}$$

(Rahman *et al.*, 2011)

Where R indicates the average of lesion radius on chilli fruit in the presence of the antagonist and C indicates the average of lesion radius on chilli fruits without the antagonist (control).

Culture Filtrate Study

Six plugs of *Trichoderma* sp. mycelia were grown in each Schott bottle containing 250 mL sterile potato dextrose broth (PDB), four replicate bottles, on a shaker at 100 rpm for 15 days at room temperature based on method described by Rahman *et al*. (2012). The culture broth was filtered twice, first through a layer of sterilised filter paper (Whatman No. 1) and then a sterilised membrane filter (Whatman, 0.22 µm). Healthy fruits of bird eye chilli were surface sterilised with 70 % ethanol for five minutes. The sterilised fruits were then washed with sterile distilled water and left to air dry on a sterilised filter paper in a laminar flow. Surface sterilised chilli fruits were then dipped in 100 ml of culture filtrate of *Trichoderma* sp. for 24 hours. Chilli fruits which served as controls were dipped in 100 mL of sterile distilled water. After 24 hours, the dipped chilli fruits were left to air dry on a sterilised filter paper in a laminar flow. A plug of *Colletotrichum* sp. culture was transferred and inserted into a wound created on each dipped

fruit using a sterilised scalpel based on method described by Nantawanit *et al*. (2010). Chilli fruits, which served as the controls, had only PDA plug inserted into the wounds. The treated chilli fruits were placed in plastic containers for each treatment, and the containers were arranged in a Completely Randomised Design. There were five replicate containers with three chilli fruits in each container, and the containers were incubated for 15 days at room temperature. Disease incidence and severity were recorded and computed using the formulae described by Ngullie *et al*. (2010) and Rahman *et al*. (2011), respectively.

Statistical Analysis

All the data were first subjected to a test for Normality. The data were then subjected to Analysis of Variance (ANOVA) if the data were normally distributed or non-parametric test (Wilcoxon signed-rank test and Mann-Whitney U test) if the data were not normally distributed. Tukey test as post-hoc test for ANOVA was used to compare the means between treatments. All statistical analysis was performed using the software SPSS version 24.

RESULTS

Pathogen Isolation and Pure Culture Establishment

Isolation of the causal agent on PDA resulted in two types of colonies. However, a dark grey colour colony with cottony mycelium and concentric rings from the middle of the colony was the most predominant on the culture plate, and therefore was selected to be subcultured for further studies. A pure culture of the predominant fungus had been successfully established, which was then used for pathogenicity test on chilli fruits and preliminary identification.

Figure 2 shows the colony morphology of the pure culture of the microorganism isolated from the naturally infected chilli fruit. As can be seen from the Figure, both upper and reverse view of the colony have dark grey colour with cottony mycelium and concentric rings from the middle of the culture plate. Microscopic observation of the isolated fungus showed setae, which had smooth-walled, tapered to

paler acute apex and slightly swollen at the base and brown in colour (Figure 3A). Hyphae of the isolated fungus were septate and conidia appeared to be a quarter moon shape, tapering towards each end with acute apex and truncate base (Figure 3B).
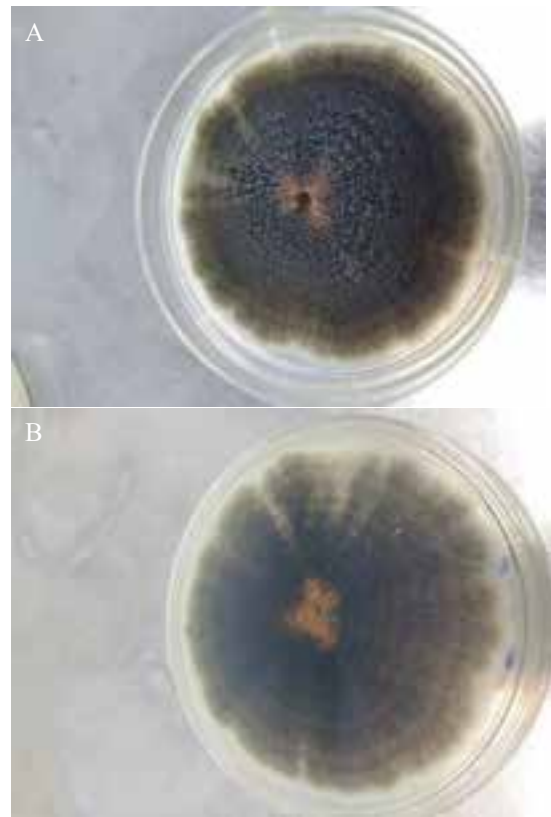

Figure 2. Colony morphology of the pure culture of the isolated fungus on PDA. (A) Upper view, (B) Reserve view.

Pathogenicity Test

All healthy chilli fruits, which were artificially inoculated with the pure culture of the isolated fungus, showed signs of white mycelial growth on the surface of the inoculated chilli fruits from second days after inoculation. Figure 4 shows disease symptoms developed on artificially inoculated and non-inoculated (controls) chilli fruits, respectively, at seven days of incubation. As can be seen from Figure 4, the chilli fruits, which were inoculated with plugs of fresh potato dextrose agar (controls), remained unaffected. However, the wounds on the artificially inoculated chilli fruits, where the mycelial discs were inserted, became black in colour. There were sunken lesions developed and black acervuli were observed on the surface
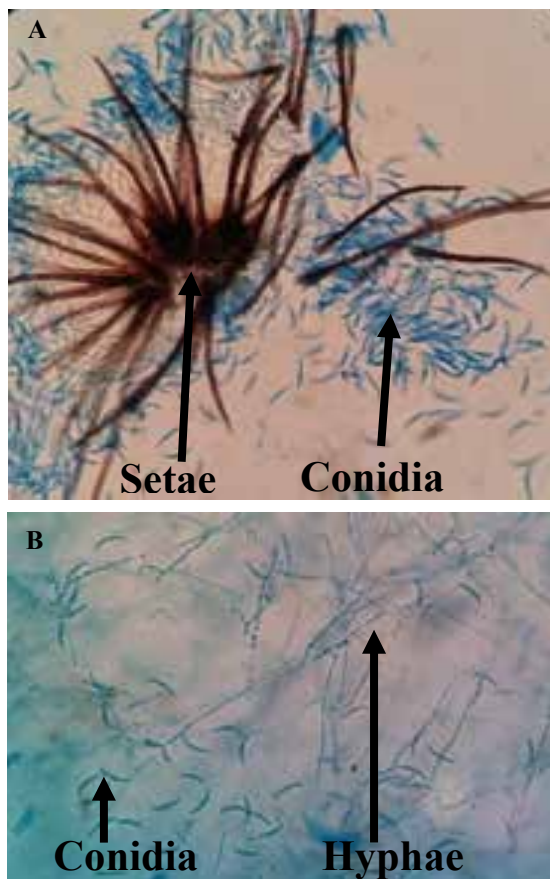
Figure 3. Microscopic structures of *C.capsici* under 400x magnification. (A) Setae and conidia, (B) Hyphae and conidia

Figure 4. Chilli fruits inoculated with PDA plugs (A; controls) and with mycelial plugs of the isolated fungus (B) on the 7th day of inoculation.

of the lesion. These symptoms were similar to those observed from the naturally infected chilli fruits, from which the pathogen was isolated. Re-isolation of the fungus from an area between infected and healthy tissue gave rise to a colony with pale grey in colour and cottony mycelium from the middle of the culture plate from the upper view.

On the reverse view, the colony was dark brown colour with concentric rings from the middle of the culture plate. Microscopic structures of the fungus (hyphae and conidia) were similar to those that were isolated from the naturally infected chilli fruits except that the hyphae were much smaller in size. The characteristics of the isolated fungus resembled to those described for *Colletotrichum capsici*, causing anthracnose disease of chilli fruits (Saxena *et al*., 2016; Shenoy *et al.*, 2007). The fungus, therefore, was preliminarily identified as a *Colletotrichum capsici*, and was confirmed to be the causal agent of the disease.
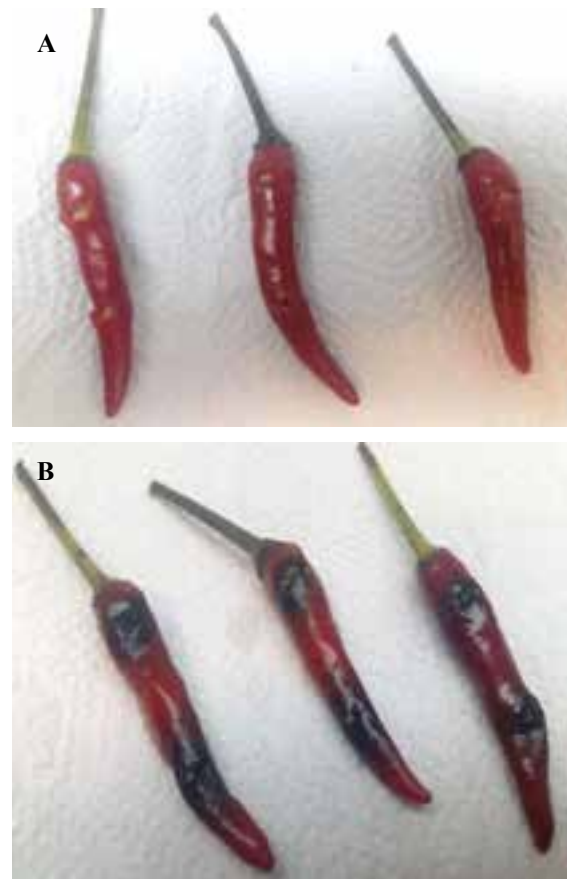
Isolation and Morphology Characteristics of Antagonistic Fungus

A *Trichoderma* sp. was successfully isolated from rhizosphere soil near the root surface of grasses. Pure culture of the isolated fungus showed characteristics of *Trichoderma* sp. based on the description by Shah *et al*. (2012). Colour from upper surface varied from whitish (Figure 5A1) to greenish (Figure 5A2) and lower surface appeared whitish (Figure 5B1) to yellowish. The conidiophore of the isolated species appeared as branches, and its conidia were round in shape (Figure 6).

Antagonistic Activity of the *Trichoderma* sp. on Culture Plate

Dual culture technique was performed to screen for antagonistic activity of *Trichoderma* sp. against *Colletotrichum capsici*. *Trichoderma* sp. grew faster as compared to *C. capsici*. Besides, the colour of *C. capsici* colony
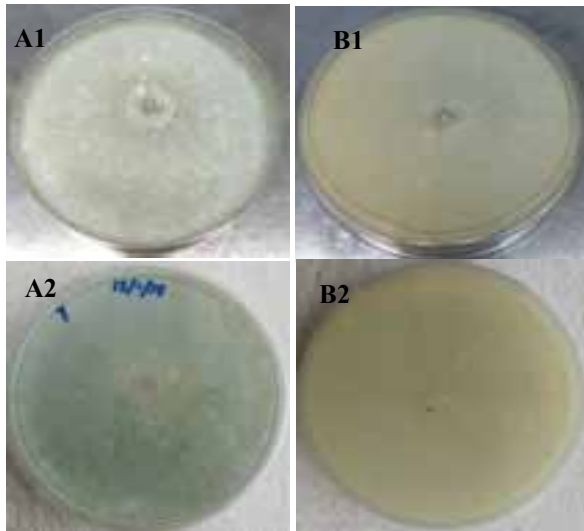
Figure 5. Colony morphology of *Trichoderma* sp. on PDA (A1, 2) Upper view, (B1, 2) Reserve view.

observed in the control plates was lighter in colour as compared to that in the treated plates (Figure 7). On the third day of inoculation, *Trichoderma* sp. started to overgrow the colony of *C. capsici*. Results from the test showed that the average growth rate of *C. capsici* was significantly ($p < 0.05$) slowed down by *Trichoderma* sp.. The average growth rate of *C. capsici* on the control plates and the treated plates was 0.94 cm and 0.85 cm, respectively.

Figure 6. Microscopic structure of *Trichoderma* sp.

under 400x magnification. Conidiophore (black dashed arrow) and conidia (black arrow).
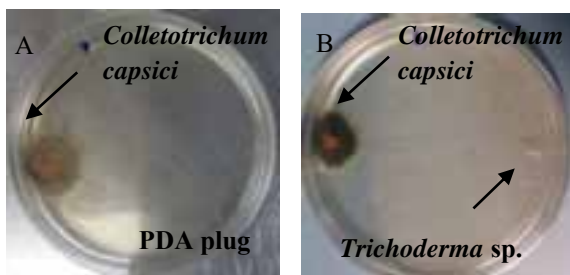

Figure 7. Colour of *Colletotrichum capsici* colony in dual culture plate on second day of incubation. (A) Control plate, (B) Treated plate.

The highest percent inhibition of radial growth of *C. capsici* recorded was on the third day of incubation (30.87%) followed by 26.83% on the first day, 18.81% on the second day and 14.80% on the fourth day. In all plates, *Trichoderma* sp. completely overgrew the colony of *C. capsici* in fourth day of incubation (Figure 8).
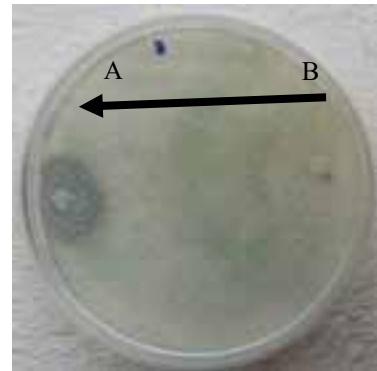

Figure 8. Growth of *Trichoderma* sp. over *Colletotrichum capsici* on a dual culture plate on 14th day of incubation. (A) *Colletotrichum capsici* colony, (B) *Trichoderma* sp. colony.

Morphology of the hyphae of *C. capsici* at the confrontation zone showed a deformed shape when observed under SEM (Figure 9). The hyphae of *C. capsici* were less turgid and seemed lysed by *Trichoderma* sp. (Figure 9b).
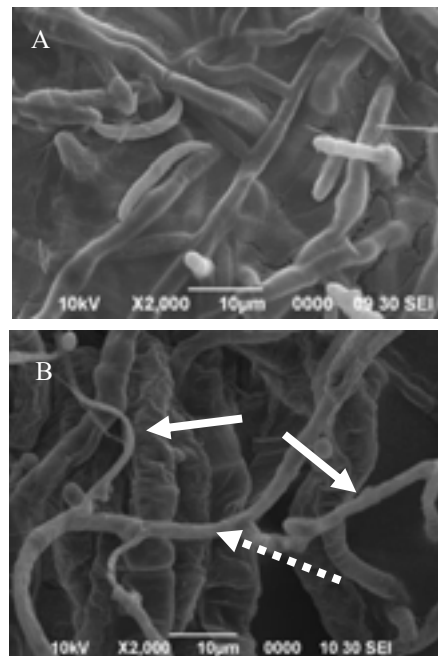

Figure 9. Change in hyphal morphology of *Colletotrichum capsici* (white arrow) when in contact with hyphae of *Trichoderma* sp. (dashed arrow) 14 days after incubation (B) under SEM with 2000x magnification as compared with control set (A).

Action of Volatile Metabolites of *Trichoderma* sp. on *Colletotrichum capsica*

Trichoderma sp. grew faster as compared to *Colletotrichum capsici*. The colour of the *C. capsici* colony observed in the Control plates was lighter as compared to that in the Treated plates (Figure 10). Besides, the growth of the colony in the Control plates was more even as that compared to the Treated plate. On the sixth day of incubation, *Trichoderma* sp. started to grow over toward the colony of *C. capsici* plated on top of the *Trichoderma* sp. plate. The result of the volatile metabolites effect of *Trichoderma* sp. showed that *Trichoderma* sp. did not significantly ($Z(6) = 1.59$, $p = 0.26$) inhibited radial growth of *C. capsici* after seven days of inoculation on PDA medium. However, growth rate of *C. capsici* in the Control plates and the Treated plates was significantly different ($p < 0.05$). The average growth rate of *C. capsici* in the Control and Treated plates was 1.08cm and 0.93cm, respectively. In all plates, *Trichoderma* sp. completely grew over onto the plate containing the colony of *C. capsici* in 14th days of incubation.
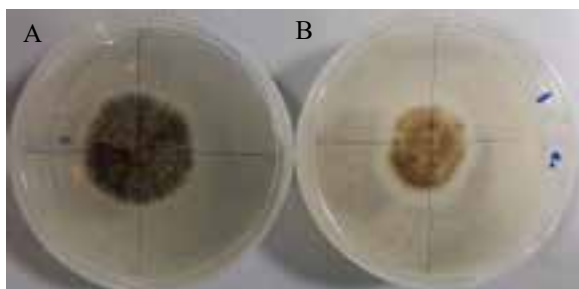
(A) Control plate, (B) Treated plate.

Figure 10. Colour of *Colletotrichum capsici* colony in volatile test on fourth day of incubation.

Table 1: Effect of *Trichoderma* sp. volatile metabolites on radial growth of *Colletotrichum capsica*.

Day	Percent inhibition of mean radial growth (%)
1	8.33 ± 6.134
2	25.95 ± 3.983
3	20.72 ± 4.467
4	17.50 ± 3.340
5	17.01 ± 3.086
6	11.57 ± 1.765
7	13.02 ± 4.091

$\pm$ standard deviation

Inhibitory Efficacy of *Trichoderma* against Anthracnose Pathogen Growth and Disease Development on Chilli Fruits

The results showed that *Trichoderma* sp. significantly ($p < 0.05$) inhibited growth and development of *Colletotrichum capsica* (Table 2). All the fruits, artificially inoculated with plugs of *C. capsici* regardless of presence of *Trichoderma* sp., showed symptoms of anthracnose. The disease incidence was therefore 100%. The symptoms observed on the fruits in this experiment were the same as those observed in the pathogenicity test (Figure 11). However, mycelia of *Trichoderma* sp. were observed to cover all over the wounded chilli fruits (Figure 11B).
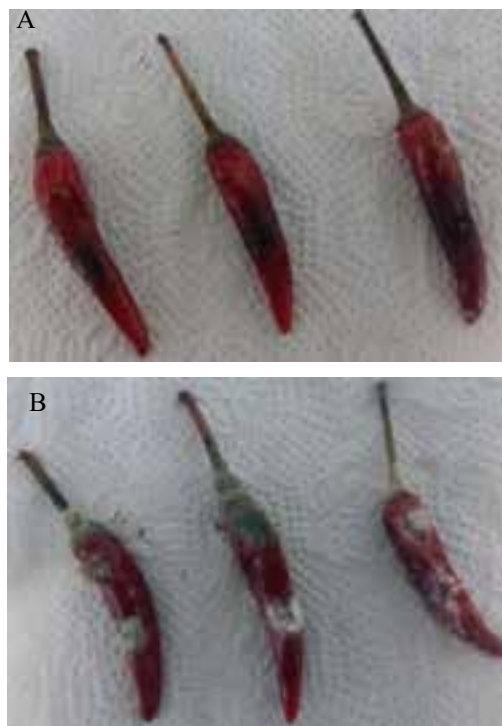

Figure 11. Diseases symptoms on chilli fruits artificially inoculated with *Colletotrichum capsici* (A) treated with PDA plugs and (B) treated with *Trichoderma* sp. plugs on fifth day after inoculation.

Table 2: Effect of *Trichoderma* sp. on disease severity caused by *Colletotrichum capsica*.

Day	Percent of disease severity (%)
2	14.11 ± 7.926
3	19.21 ± 6.036
4	64.35 ± 22.082
5	50.09 ± 19.527

$\pm$ standard deviation

Culture Filtrate Study

The results indicated that the culture filtrates of *Trichoderma* sp. significantly inhibited the growth of *Colletotrichum capsici* and the development of anthracnose disease on the artificially inoculated chilli fruits (Table 3). There was 100% of disease incidence, and the symptoms that were developed on the fruits in this experiment were the same as those observed in the pathogenicity test (Figure 12).
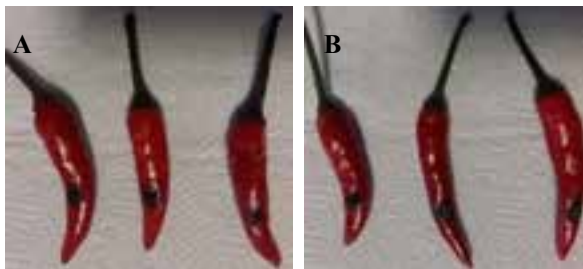

Figure 12. Culture filtrate of *Trichoderma* sp. on wounded chilli fruits with *Colletotrichum capsici*. (A) Control treatment, (B) Treated treatment.

DISCUSSION

Anthracnose is one of the major causes of economic loss to chilli production (Than *et al*., 2008a; Than *et al*., 2008b). Chemicals have been used to control the anthracnose of chilli but resistance has been developed in the pathogen of anthracnose (Benítez *et al*., 2004). In order to reduce the usage of the chemicals on the control of chilli anthracnose pathogen, alternative control approaches are needed (Rahman *et al*., 2011). Antagonists, soil microorganisms that can interfere with pest's activities such as *Trichoderma* spp. are one of the popular fungi known for their antagonism (Chernin & Chet, 2002; Verma *et al*., 2007).

The pathogen associated with the naturally infected chilli was successfully isolated out and proved to be pathogenic. The pathogen was preliminarily identified as *Colletotrichum capsici* since it had similar characteristics of dark grey colony to that described for *C. capsici* by Liu *et al.* (2016), of septate hyphae, the quarter moon shape of the conidia, and the presence of setae as those described for *C. capsici* by Shenoy *et al.* (2007). However, it is worthwhile to have a molecular analysis to confirm the species.

Table 3: Effect of *Trichoderma* sp. culture filtrate on disease severity caused by *Colletotrichum capsici*

Day	Percent of disease severity (%)
2	18.51 ± 4.383
3	37.14 ± 5.389
4	31.70 ± 11.612
5	55.00 ± 13.845

The antagonist that successfully isolated out was identified as a *Trichoderma* sp.. The green colony characteristics observed in the present study were in agreement with those described in the studies by Shah *et al*. (2012). The branching conidiophore and round and green colour conidia of the isolated *Trichoderma* sp. in the present study were similar to those described in the study by Armando *et al*. (2017).

In the present study, *Trichoderma* sp. reduced the mycelia growth of *Colletotrichum capsici* in dual culture assay. The mechanism observed in the dual culture assay by *Trichoderma* sp. might be the competition for space and nutrients between the pathogen and the antagonist (Amin *at al*., 2010b) and lysis (Begum *et al*., 2008). Growth of *Trichoderma* sp. over *C. capsici* was also observed in the study by Amin *et al*. (2010a) and Sawant (2014). In addition, Intana *et al*. (2007), when examined the efficacy of three mutant and two wild type strain of *T. harzianum* in inhibiting mycelia growth of *C. capsici*, also reported that all the strains of *Trichoderma* were able to inhibit and overgrow the colony of *C. capsici*. A similar study by Begum and Nath (2015) indicated that *T. harzianum* isolate Th-2 was effective on inhibiting the mycelia growth of all isolates of *C. capsici* where the highest percent that been observed was 100% inhibition. However, in the present study, the inhibition of mycelial growth of *C. capsici* by the *Trichoderma* sp. was only 30.87%.

Previous study reported inhibitory mechanisms by *Trichoderma virens* and *Trichoderma harzianum* to *C. truncatum* through competition, parasitism and antibiosis (Begum *et al*., 2008). However, in this study, coiling and penetration of *Trichoderma* sp. were not observed in the SEM analysis. The *Trichoderma* sp. grew over the colony of *C. capsici* and at the point where the two fungi encountered, mycelia of *C. capsici* was found to

change in turgidity. This change in shape might be attributed to the ability of *Trichoderma* sp. to secrete enzymes such as chitinase and glucanase which were reported in the study by Alka *et al*. (2017). According to Cuervo-Parra *et al*. (2011), deformation and disorganization of cell wall structure of *Moniliophthora rorer* which became rough was due to antifungal substances secretion of *Trichoderma* such as enzymes and antibiotics. In addition, the change in the shape of hyphae of *C. capsici* by *Trichoderma* sp. which became rough in structure in this study might also be due to lysis (Shahbazi *et al*., 2014). A study conducted by Palaniyandi *et al*. (2013) also recorded the occurrence of lysis on the fungal mycelia of *C. coccodes* by *Streptomyces phaeopurpureus*.

In this study, reduced growth of *C. capsici* in the volatile metabolite experiment illustrated the ability of the *Trichoderma* sp. to produce volatile metabolites. This mechanism has been reported for the *T. virens* and *T. harzianum* by Amin *et al*. (2010b) to control the mycelial growth of *C. capsici* by more than 50% and the radial growth was 12.73 mm and 13.41 mm respectively. Waterhouse (1968) also reported that metabolites produced by *Trichoderma* sp. was effective against *C. gloeosporioides*. Besides, *Trichoderma* sp. produced volatile metabolites which suppressed the growth of *Pythium aphanidermatum* (Muthukumar *et al*., 2011). Well known volatiles produced by *Trichoderma* spp. are trichodermin and trichodermol which are able to degrade cell wall of pathogens (Elad, 2000).

In vivo study was implemented to examine or verify whether the isolated Trichoderma would be effective in natural condition. In the presence of *Trichoderma* sp., disease severity caused by *C. capsici* was reduced in the present study. Similarly, in the study by Vasanthakumari and Shivanna (2014), *T. harzianum* decreased incidence and severity of the disease caused by *C. graminicola* in sorghum. The growth of the *Trichoderma* sp. on the surface of the wounded chilli fruits in this study might be due to the ability of *Trichoderma* sp. to colonize on the fruit surface (Ippolito & Nigro, 2000) and niche overlap between competitors is required to perform successful colonization (Kinkel & Lindow, 1997). However, the presence of the *Trichoderma* mycelia on chilli fruits would affect the visual appearance of the fruits, which in turn would reduce its acceptability by consumers. Therefore, culture filtrate of *Trichoderma* sp. was used in a subsequent *in vivo* experiment.

Significant suppression in the growth of *C. capsici* and the development of anthracnose disease on the artificially inoculated chilli fruits by the culture filtrates of *Trichoderma* sp. suggested that the isolated *Trichoderma* sp. produced substances which might have antifungal effect. Shi *et al*. (2012) reported the production of antimicrobial peptides by *T. pseudokoningii* again a number of plant fungal pathogens while Vinale *et al*. (2014) discussed various secondary metabolites produced by *Trichoderma* genus, which are toxic to phytopathogens. *Trichoderma* spp. were also reported to produce many cell wall degrading enzyme such as xylanase and chitinase (Pandey *et al*., 2015). The finding in the present study agree with the study conducted by Rahman *et al*. (2012) who also found that application of culture filtrate of *T. harzianum* significantly decreased the disease severity caused by *C. capsici*. In addition, Rahman *et al*. (2013) reported that 30 day old culture filtrates of all *Trichoderma* strains in their study significantly reduced percentage of anthracnose disease severity on chilli fruits. According to Padder and Sharma (2011), *Trichoderma viride* has the best potential to inhibit mycelia growth and germination of spore of *Colletotrichum lindemuthianum*. Finally, higher percent inhibition (64%) of *Trichoderma* sp. toward *C. capsici* when *Trichoderma* plugs were used compared to 55% inhibition when culture filtrate was applied in this study indicated that, apart from antifungal effects, competition for space and nutrients could be another antagonism mechanism employed by *Trichoderma* sp. to inhibit the growth of *C. capsici* and the anthracnose development on the artificially infected fruits.

CONCLUSION

The isolated *Trichoderma* sp. has the potential as a biological control agent for *Colletotrichum capsici*, the pathogen of chilli anthracnose. It inhibited the growth of the pathogen on culture and reduced the disease severity on the chilli

fruits. For post-harvest treatment, application of a culture filtrate of the *Trichoderma* sp. would be more appropriate to avoid the growth of the antagonist on the fruit surface, which will reduce the product appearance, thus market quality of the chilli fruits. However, in the field, the antagonist might be used in either form, with and without mycelia. In this study, the antagonistic ability of the isolated *Trichoderma* sp. was only tested in the laboratory or a controlled environment, so it would be worthwhile to examine the effect of the isolated *Trichoderma* sp. in the field conditions.

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REFERENCES

Alka, Patil, R.K. & Prajapati, B.K. (2017). Effect of *Trichoderma* spp. and its culture filtrate antagonists on growth and management of *Rhizopus* rot of tomato fruit in vitro and in vivo. *Journal of Pharmacognosy and Phytochemistry,* 6(4): 394-398.

Amin, F., Razdan, V.K., Mohiddin, F.A., Bhat, K. A. & Saba, B. (2010a). Potential of *Trichoderma* species as biocontrol agents of soil borne fungal propagules. *Journal of Phytology*, 2(10): 38-41.

Amin, F., Razdan, V.K., Mohiddin, F.A., Bhat, K.A. & Sheikh, P.A. (2010b). Effect of volatile metabolites of *Trichoderma* species against seven fungal plant pathogens in-vitro. *Journal of Phytology*, 2(10): 34-37.

Armando, N.G., Marfetán, J.A. & Folgarait, P. J. (2017). *Trichoderma* species associated with acromyrmex ant nests from Argentina and first report of *Trichoderma lentiforme* for the country. *Darwiniana*, 5(1): 72-82.

Begum, M.M., Sariah, M., Abidin, Z.M.A., Puteh, A.B. & Rahman, M.A. (2008). Antagonistic potential of selected fungal and bacterial biocontrol agents against *Colletotrichum truncatum* of soybean seeds. *Pertanika Journal of Tropical Agriculture Science*, 31: 45-53.

Begum, S. & Nath, P.S. (2015). Eco-friendly management of anthracnose of chilli caused by *Colletotrichum capsici*. *Journal of Applied and Natural Science,* 7(1): 119 – 123.

Benítez, T., Rincón, A.M., Limón, M.C. & Codón, A.C. (2004). Biocontrol mechanisms of *Trichoderma* strains. *International Microbiology*, 7: 249-260.

Bharathi, R., Vivekananthan, R., Harish, S., Ramanathan, A. & Samiyappan, R. (2004). Rhizobacteria-based bio-formulations for the management of fruit rot infection in chillies. *Crop Protection*, 23(9): 835-843.

Carvalho, D.D.C., Junior, M.L., Martins, I., Inglis, P.W. & Mello, S.C. (2014). Biological control of *Fusarium oxysporum* f. sp. *phaseoli* by *Trichoderma harzianum* and its use for common bean seed treatment. *Tropical Plant Pathology,* 39(5): 384-391.

Chernin, L. & Chet, I. (2002). Microbial enzymes in biocontrol of plant pathogens and pests, Marcel Dekker Incorporate, New York, N.Y., 171-225.

Choi, Y.W., Hyde, K.D., & Ho, W.W H. (1999). Single spore isolation of fungi. *Fungal Diversity,* 3: 29-38.

Cuervo-Parra, J.A., Ramírez-Suero, M., Sánchez-López, V. & Ramírez-Lepe, M. (2011). Antagonistic effect of *Trichoderma harzianum* VSL291 on phytopathogenic fungi isolated from cocoa (*Theobroma cacao* L.) fruits. *African Journal of Biotechnology*, 10(52): 10657-10663.

Elad, Y. (2000). Biological control of foliar pathogens by means of *Trichoderma harzianum* and potential modes of action. *Crop Protection,* 19: 709-714.

Hill, R.A., Ambrose, A., Sajali, N.A., Yatim, M., Valdez, R.B., Agbayani, F., Bungang, J., Minchin, R.F. & Stewart, A. (2010). Bioprotection of *Acacia mangium* using *Trichoderma* in Malaysia. *Microbial Products: Exploiting microbial diversity for sustainable plant production (eds Zydenbos SM & Jackson TA). New Zealand Plant Protection Society Incorporated*, 51-55.

Intana, W., Suwanno, T., Chamswarng, C., Chantrapromma, K. & Ngamriabsakul, C. (2007). Increased efficacy for controlling

anthracnose of chili using antifungal metabolites from mutant strains of *Trichoderma harzianum*. *Thai Journal of Agricultural Science,* 40(1-2): 65-72.

Ippolito, A. & Nigro, F. (2000). Impact of preharvest application of biological control agents on postharvest diseases of fresh fruits and vegetables. *Crop Protection,* 19: 715-723.

Karim, M.S.A., Rashid, S.S.A., Adzahan, N.M. & Camillo, A. A. (2011). Consumers' Perspective Towards Malaysian Traditional Food: Sambal Belacan (Chilli Shrimp Paste), A Preliminary Investigation. *Journal of Agribusiness Marketing,* 4: 68-92.

Kim, H.J., Lee, E.J., Park, S.H., Lee, H.S. & Chung, N. (2014). Biological control of anthracnose (*Colletotrichum gloeosporioides*) in pepper and cherry tomato by *Streptomyces* sp. A1022. *Journal of Agricultural Science*, 6(2): 54.

Kinkel, L.L. & Lindow, S.E. (1997). Microbial competition and plant disease biocontrol. In: Andow, D. A., Ragsdale, D. W., Nyvall, R. F. (Eds.), *Ecological Interaction and Biological Control*. Westview Press, Boulder, CO, USA, 128-138.

Liu, F., Tang, G., Zheng, X., Li, Y., Sun, X., Qi, X., Zhou, Y., Xu, J., Chen, H., Chang, X. & Zhang, S. (2016). Molecular and phenotypic characterization of *Colletotrichum* species associated with anthracnose disease in peppers from Sichuan Province, China. *Scientific Reports*, 6: 32761.

Montri, P., Taylor, P.W.J. & Mongkolporn, O. (2009). Pathotypes of *Colletotrichum capsici*, the causal agent of chili anthracnose, in Thailand. *Plant Disease,* 93: 17-20.

Muthukumar, A., Eswaran, A. & Sanjeevkumas, K. (2011). Exploitation of *Trichoderma* species on the growth of *Pythium aphanidermatum* in chilli. *Brazilian Journal of Microbiology,* 42: 1598-1607.

Naipagropediaraichur. (2012). *Chilli Anthracnose*. Retrieved August 24, 2017 from http://agropedia.iitk.ac.in/content/chilli-anthracnose

Nantawanit, N., Chanchaichaovivat, A., Panijpan, B. & Ruenwongsa, P. (2010). Induction of defense response against *Colletotrichum capsici* in chili fruit by the yeast *Pichi guilliermondii* strain R13. *Biological Control,* 52: 145-152.

Ngullie, M., Daiho, L. & Upadhyay, D.N. (2010). Biological management of fruit rot in the world's hottest chilli (*Capsicum chinense* Jacq.). *Journal of Plant Protection Research*, 50(3): 269-273.

Padder, B.A. & Sharma, P.N. (2011). *In vitro and in vivo* antagonism of biocontrol agents against *Colletotrichum lindemuthianum* causing bean anthracnose. *Archives of Phytopathology and Plant Proctection,* 44(10): 961-969.

Palaniyandi, S.A., Yang, S.H. & Suh, J.W. (2013). Extracellular proteases from *Streptomyces phaeopurpureus* ExPro138 inhibit spore adhesion, germination and appressorium formation in *Colletotrichum coccodes*. *Journal of Applied Microbiology,* 115: 207-217.

Pandey K.K. & Pandey P.K. (2003). Survey and surveillance of vegetable growing areas for prevalence of major diseases. *Journal of Vegetation Science,* 30(20): 128-134.

Pandey, S., Srivastava, M., Shahid, M., Kumar, V., Singh, A., Trivedi, S. & Srivastava, Y.K. (2015). *Trichoderma* species cellulases produced by solid state fermentation. *Journal of Data Mining in Genomics & Proteomics*, 6(2): 170.

Rahman, AM., Rahman, M. M., Azad, K.A. & Alam, F.M. (2011). Inhibitory effect of different plant extracts and antifungal metabolites of *Trichoderma* strains on the conidial germination and germ tube growth of *Colletotrichum capsici* causing chili anthracnose. *International Journal of Agronomy and Agricultural Research,* 1(1): 20-28.

Rahman, M.A., Rahman, M.M., Kamruzzaman, M., Begum, M.F. & Alam, M.F. (2012). Use of culture filtrates of *Trichoderma* strains as a biological control agent against *Colletotrichum capsic i* causing anthracnose fruit rot disease of chili. *Journal of Biodiversity and Environmental Sciences,* 2(1): 9-18.

Rahman, M.A., Razvy, M.A. & Alam, M.F. (2013). Antagonistic activities of *Trichoderma* strains against chili anthracnose pathogen. *International Journal of Microbiology and Mycology,* 1(1): 7-22.

Ratanacherdchai, K., Wang, H.K., Lin, F.C. & Soytong, K. (2010). ISSR for comparison of cross-inoculation potential of *Colletotrichum capsici* causing chilli anthracnose. *African Journal of Microbiology Research*, 4(1): 76-83.

Rosli, N.A. (2017). *Antimicrobial properties of Manihot esculenta, Cymbopogon citratus and Mentha piperita against crop pathogens.* (Doctoral dissertation, UNIMAS).

Sangdee, A., Sachan, S. & Khankhum, S. (2011). Morphological, pathological and molecular variability of *Colletotrichum capsici* causing anthracnose of chilli in the North-east of Thailand. *African Journal of Microbiology Research*, 5(25): 4368-4372.

Sawant, I.S. (2014). *Trichoderma*- foliar pathogen interactions. *The Open Mycology Journal,* 8: 58-70.

Saxena, A., Raghuwanshi, R., Gupta, V.K. & Singh, H. B. (2016). Chilli Anthracnose: The Epidemiology and Management. *Frontiers in Microbiology*, 7: 1527.

Shah, S., Nasreen, S. & Sheikh, P.A. (2012). Cultural and morphological characterization of *Trichoderma* spp. associated with green mold disease of *Pleurotus* spp. in Kashmir. *Research Journal of Microbiology*, 7(2): 139-144.

Shahbazi, P., Musa, M.Y., Tan, G.Y.A., Avin, F. A., Teo, W.F.A. & Sabaratnam, V. (2014). *In vitro* and *in vivo* evaluation of *Streptomyces* suppressions against anthracnose in chili caused by *Colletotrichum*. *Sains Malaysiana,* 43(5): 697-705.

Shenoy, B.D., Jeewon, R., Lam, W.H., Bhat, D. J., Than, P.P., Taylor, P.W.J. & Hyde, K.D. (2007). Morpho-molecular characterization and epitypification of *Colletotrichum capsici* (*Glomerellaceae, Sordariomycetes*), the

causative agent of anthracnose in chilli. *Fungal Diversity,* 27: 197-211.

Shi, M., Chen, L., Wang, X. W., Zhang, T., Zhao, P.B., Song, X.Y., Sun, C.Y., Chen, X.L., Zhou, B.C. & Zhang, Y.Z. (2012). Antimicrobial peptaibols from *Trichoderma pseudokoningii* induce programmed cell death in plant fungal pathogens. *Microbiology,* 158: 167-175.

Than, P.P., Prihastuti, H., Phoulivong, S., Taylor, P.W. & Hyde, K.D. (2008a). Chilli anthracnose disease caused by *Colletotrichum* species. *Journal of Zhejiang University Science B*, 9(10): 764-778.

Than, P.P., Jeewon, R., Hyde, K.D., Pongsupasamit, S., Mongkolporn, O. & Taylor, P.W.J. (2008b). Characterization and pathogenicity of *Colletotrichum* species associated with anthracnose disease on chilli (*Capsicum* spp.) in Thailand. *Plant Pathology*, 57(3): 562-572.

Vasanthakumari, M.M. & Shivanna, M.B. (2014). Biological control of sorghum anthracnose with rhizosphere and rhizoplane fungal isolates from perennial grasses of the Western Ghats of India. *European Journal of Plant Pathology,* 139: 721–733.

Verma, M., Brar, S.K., Tyagi, R.D., Surampalli, R.Y. & Valero, J.R. (2007). Antagonistic fungi, *Trichoderma* spp.: Panoply of biological control. *Biochemical Engineering Journal*, 37(1): 1-20.

Vinale, F., Sivasithamparam, K., Ghisalberti, E. L., Wool, S. L., Nigro, M., Marra, R., Lombardi, N., Pascale, A., Ruocco, M., Lanzuise, S., Manganiello, G. & Lorito, M. (2014). *Trichoderma* secondary metabolites active on plants and fungal pathogens. *The Open Mycology Journal*, 8, (Suppl-1, M5): 127-139.

Waterhouse, G.M. (1968). The genus *Pythium* Pringsheim. *Mycological Papers,* 110: 1-50.

Williams, C.N., Uzo, J.O. & Peregrine, W.T.H. (1991). *Vegetable Production in the Tropics*. England: Longman Scientific & Technical. P 179.

SHORT COMMUNICATION

Antifungal Properties of Selected Medicinal Plant Species Against *Fusarium* spp. – A Preliminary Study

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ABSTRACT

Usage of synthetic fungicides has inevitably been one of the agricultural practices in combating crop pathogens and maintaining the quality of production. Although fungicides have been proven to be profoundly effective, excessive and frequent reliance on these synthetic fungicides have caused negative impacts to the environment and human health. Besides that, indiscriminate use of fungicides may lead to the development of resistant strains of pathogenic fungi. The need to find an alternative solution to synthetic fungicides has led to the interest in finding plant-based fungicides. This study aimed to test the antifungal properties of plant extracts from 13 different medicinal plant species towards plant pathogenic fungi. Absolute methanol was used as a solvent to extract the secondary metabolites from the different plant species. The effect of methanolic crude extract at different concentrations (500 µg/ml, 250 µg/ml and 100 µg/ml), from different medicinal plant species, were tested on the growth of two *Fusarium* spp., *Fs*B and *Fs*P. The assay showed that the methanolic crude extract from six plant species *viz. Alpinia galanga*, *Annona muricata*, *Archidendron jiringa*, *Nephelium lappaceum*, *Polygonum minus* and *Artocarpus* hybrid (Nanchem) had successfully inhibit the radial mycelial growth of either *Fs*B or *Fs*P, or both. The assay suggested that the six plant species have antifungal properties towards the crop pathogenic fungi tested.

Keywords: antimicrobial, *Fusarium*, plant extracts, methanolic extracts, biofungicides

Combating plant pathogens has always been a constant struggle for farmers to obtain high yield with good quality produce. Agricultural practices such as application of synthetic fungicides are commonly used to reduce diseases (Brent & Holloman, 2007). However, the excessive usage and frequent reliance of synthetic fungicides, have led to several other problems such as toxicity towards the environment due to the residues of synthetic fungicides (Wightwick *et al.*, 2010), threatening the functionality of human reproductive system by the active compounds (Peraica *et al.,* 1999; Hossain *et al.*, 2010) and emergence of resistant strains. These may cause more severe diseases and outbreaks (Francis & Keinath, 2010). Hence, there is a need to produce a safer and biodegradable fungicides derived from natural resources (Brito-Argaez *et al.*, 2009).

Plant-based compounds are one of the potential resources for production of safer and biodegradable fungicides. Secondary metabolites from plants such as alkaloids, terpenoids, cyanogenic, glucosides, and phenolics are groups of compounds known to be involved in plant defence mechanisms (Bennett & Wallsgrove, 1994; Bravo, 1998; Balasundram *et al.*, 2006). These compounds are the possible candidates for developing biofungicides. For instance, phenolic compounds such as luteolin-7-glucoside, oleuropein, rutin, and tyrosol in olive plants were reported to possess antimicrobial effect against selected pathogens (Báidez *et al.*, 2007; Pereira *et al*., 2007). The present study aimed to test the antifungal properties of methanolic crude extracts from 13 plant species against two *Fusarium* spp., a common plant pathogenic fungus. Table 1 shows the list of

Table 1. The plant parts used for extraction in this study for the 13 medicinal plant species.

Plant parts	Plant species	Common name
Leaves	*Annona muricata* (L.)	Durian Belanda
	Archidendron jiringa (Jack)	Jering
	Artocarpus sp. hybrid (Nanchem)	Cempedak
	Centella asiatica (L.)	Pegaga
	Cymbopogon citratus (D.C.) Stapf	Serai
	Manihot esculenta (Crantz)	Ubi kayu
	Mentha piperita (L.)	Pudina
	Nephelium lappaceum (L.)	Rambutan
	Oenanthe javanica (Blume)	Selom
	Polygonum minus (Huds)	Kesum
	Piper betle (L.)	Sireh
Rhizome	*Alpinia galanga* (L.) Willd.	Lengkuas
	Zingiber officinale Rosc.	Halia

plant species and the plant parts used in this study.

Fresh and disease-free leaves from 11 medicinal plant species were collected from different residential areas or were bought from Medan Niaga Satok, Sarawak. The leaves were rinsed with tap water to remove contaminants. The leaves were patted dry and air dried for seven days without direct sunlight, at room temperature. Fresh and disease-free rhizomes from the other two medicinal plant species (*Alpinia galanga* and *Zingiber officinale*) were also rinsed with tap water and patted dry. They were then cut into smaller pieces and air dried for two weeks without direct sunlight at room temperature.

The air-dried samples were homogenized into powder form using a blender. A total of 150 grams of the powdered samples were soaked in pure methanol and shaken overnight at room temperature. The extracts were filtered using Whatman filter paper No. 1 the following day. The methanolic extract from each plant species was then concentrated using a rotary evaporator. The water bath of rotary evaporator was set at 45°C ± 2.0. The concentrated crude extract was weighed and the yield of crude extract was calculated. In average, the yield of methanolic

crude extract from the 13 medicinal plant species was between 4 – 14%.

The antifungal assay was adapted and modified from CSLI (2012). Concentrated crude extract was dissolved with Dimethyl sulfoxide (DMSO) and filter sterilized using a micro filter (0.22 μm). Media was prepared by mixing equal volume of methanol crude extract (from different stock concentrations) into PDA (Sigma-Aldrich) to obtain final concentrations of 500 μg/ml, 250 μg/ml, and 100 μg/ml. Control was prepared by mixing DMSO (same volume as the crude extract) into PDA. The concentration of DMSO in the media was kept at 1%.

Two fungal species were selected for this study, namely *Fusarium* sp. isolated from banana (*Fs*B) and *Fusarium* sp. isolated from black pepper (*Fs*P). The pathogenic fungi were provided by Semongok, Agriculture Research Centre (ARC). Plugs of fungi (6 mm Ø) used in the antifungal assay were obtained from pure cultures of six to seven days old. The antifungal assay had ten replicates per treatment for each plant-fungal isolate pair. All plates were incubated at room temperature. The radial mycelial growth of the fungus was scored daily by measuring the diameter of the mycelia at

the back of Petri dish. The average fungal growth rate was calculated. One-way ANOVA was used to analyse the data. Methanolic crude extract from six plant species showed significant effects on the growth rate of *Fusarium* spp. tested. Methanolic crude extract of *Annona muricata* (L.), *Alpinia galanga* (L.) Willd., and *Nephelium lappaceum* (L.) retarded the growth of both

*Fs*B and *Fs*P, whereas methanolic crude extract from *Archidendron jiringa* (Jack), *Artocarpus* sp. hybrid (Nanchem), and *Polygonum minus* (Huds) had slowed down the growth of either *Fs*B or *Fs*P, while the methanolic crude extract of the other medicinal plant species had no effect towards the growth rate of *Fusarium* spp. tested (Table 2).

Table 2. Average growth rate (cm/day) of the two *Fusarium* spp. on different concentrations of methanol crude extract from the 13 plant species.

Plant species	Fungal isolate	Growth rate (cm/day)*			
		Control	100 µg/ml	250 µg/ml	500 µg/ml
A. galanga	*Fs*B	0.36[a]	0.37[a]	0.32[b]	0.28[c]
	*Fs*P	0.48[a]	0.46[a]	0.45[a]	0.40[b]
A. muricata	*Fs*B	0.70[a]	0.66[a]	0.65[b]	0.68[c]
	*Fs*P	0.82[a]	0.73[b]	0.71[c]	0.68[d]
A. jiringa	*Fs*B	0.64[a]	0.69[a]	0.66[a]	0.59[a]
	*Fs*P	0.74[a]	0.75[b]	0.76[c]	0.66[d]
Artocarpus sp. hybrid (Nanchem)	*Fs*B	0.61[a]	0.60[a]	0.59[a]	0.55[b]
	*Fs*P	0.84[a]	0.89[a]	0.85[a]	0.82[a]
C. asiatica	*Fs*B	0.40[a]	0.40[a]	0.40[a]	0.39[a]
	*Fs*P	0.33[a]	0.37[a]	0.37[a]	0.41[a]
C. citratus	*Fs*B	0.77[a]	0.87[a]	0.79[a]	0.79[a]
	*Fs*P	1.14[a]	1.06[a]	1.10[a]	1.13[a]
M. esculenta	*Fs*B	0.94[a]	1.06[a]	1.09[a]	0.92[a]
	*Fs*P	2.06[a]	2.23[a]	2.28[a]	2.12[a]
M. piperita	*Fs*B	0.73[a]	0.75[a]	0.78[a]	0.77[a]
	*Fs*P	1.45[a]	1.50[a]	1.60[a]	1.61[a]
N. lappaceum	*Fs*B	0.42[a]	0.38[a]	0.34[b]	0.32[c]
	*Fs*P	0.45[a]	0.43[b]	0.40[c]	0.37[d]
O. javanica	*Fs*B	0.80[a]	0.98[a]	0.94[a]	0.87[a]
	*Fs*P	0.66[a]	0.89[a]	0.87[a]	0.83[a]
P. minus	*Fs*B	0.38[a]	0.39[a]	0.48[a]	0.39[a]
	*Fs*P	0.39[a]	0.41[a]	0.39[a]	0.35[b]
P. betle	*Fs*B	0.60[a]	0.55[a]	0.61[a]	0.59[a]
	*Fs*P	0.87[a]	0.89[a]	1.09[a]	0.85[a]
Z. officinale	*Fs*B	0.75[a]	0.71[a]	0.73[a]	0.78[a]
	*Fs*P	0.74[a]	0.93[a]	0.69[a]	0.67[a]

*Different letters indicate significant difference of fungal growth rate on media infused with different concentrations of crude extract ($p < 0.05$).

In general, the methanolic crude extract from the six plant species managed to retard the fungal growth by a delay of one to three days to reach full plate (8 cm Ø) as compared to the corresponding control plates (Table 3).

The inhibition percentage of the fungal growth by the different concentrations of methanolic crude extract were small for both *Fusarium* spp. and negligible, therefore it is not presented.

Table 3. The significant fungal growth delay on the plates containing methanolic crude extract from the six medicinal plant species having antifungal properties.

Plant species	Fungal isolates	Effective concentrations	Number of days
A. galanga	*Fs*B	500 µg/ml	2-3 days
		250 µg/ml	1 day
	*Fs*P	500 µg/ml	2-3 days
A. muricata	*Fs*B	250 µg/ml	1 day
	*Fs*P	ALL	1 day
A. jiringa	*Fs*P	500 µg/ml	1 day
N. lappaceum	*Fs*B	500 µg/ml	2-3 days
		250 µg/ml	2-3 days
	*Fs*P	500 µg/ml	2-3 days
		250 µg/ml	2-3 days
		100 µg/ml	1 day
P. minus	*Fs*P	500 µg/ml	1 day
Artocarpus sp. hybrid (Nanchem)	*Fs*B	500 µg/ml	1 day

Although the inhibition effect may be negligible, the success of retarding the fungal growth is an indication for the presence of antifungal compounds in the plant methanolic crude extract against *Fs*B and *Fs*P. Previous phytochemical studies on methanolic extracts of *A. galanga*, *A. muricata, A. jiringa*, *N. lappaceum*, and *P. minus* reported potential compounds in the plant extract that possess antimicrobial properties in the respective studies (Table 4).

There is a possibility that the compounds reported (Table 4) is also present in the crude extract of the current study. Against *Fs*B and *Fs*P, these compounds may not have strong effect but only manage to slow down the fungal growth. So far, there is no phytochemical studies on Nanchem. It is possible that the methanolic crude extract of Nanchem contain polar compounds (Cowan, 1999) with antifungal properties.

Table 4. Class of compounds detected in the methanolic extract of the six medicinal plant species having antifungal properties identified.

Plant species	Classes of compounds	References
A. galanga	Phenols Flavonoids ie. 1'- Acetoxychavicol acetate (ACA) and galangin	Seo *et al.* (2013) Nuttaporn (2007)
A. muricata	Alkaloids Flavonoids Tannins Terpenoids Polyphenols	Chauhan & Mittu (2015) George *et al.* (2015)
A. jiringa	Terpenoids Tannins Polyphenols	Cowan (1999)
N. lappaceum	Phenols	Thitilertdecha *et al.* (2008)
P. minus	Phenols Flavonoids	Qader *et al.* (2012)
Artocarpus sp. hybrid (Nanchem)	N/A	-

To conclude, this preliminary study showed that six of the 13 medicinal plant species used in this study *viz*. *A. muricata, A. jiringa, A. galanga,* Nanchem*, N. lappaceum* and *P. minus*, have antifungal property against *Fs*B and *Fs*P. The methanolic crude extract, at high concentration, managed to retard the fungal growth. The inhibition percentage was, however, negligible. Future studies can be conducted by using different parts of the plant species to screen for antifungal properties.

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REFERENCES

Báidez, A.G., Gómez, P., Del Río, J.A. & Ortuño, A. (2007). Dysfunctionality of the xylem in *Olea europaea* L. plants associated with the infection process by *Verticillium dahliae* Kleb. Role of phenolic compounds in plant defense mechanism. *Journal of Agricultural and Food Chemistry*, 55(9): 3373-3377.

Balasundram, N., Sundram, K. & Samman, S. (2006). Phenolic compounds in plants and agri-industrial by-products: Antioxidant activity, occurrence, and potential uses. *Food Chemistry*, 99(1): 191-203.

Bennett, R.N. & Wallsgrove, R.M. (1994). Secondary metabolites in plant defence mechanisms. *New Phytologist*, 127(4): 617-633.

Bravo, L. (1998). Polyphenols: chemistry, dietary sources, metabolism, and nutritional significance. *Nutrition Reviews*, 56(11): 317-333.

Brent, K.J. & Holloman, D.W. (2007). *Fungicide resistance in crop pathogens: How can it be managed?* Fungicide Resistance Action Committee, Monograph 1, Global Crop Protection Federation.

Brito-Argáez, L., Moguel-Salazar, F., Zamudio, F., González-Estrada, T. & Islas-Flores, I. (2009). Characterization of a *Capsicum chinense* seed peptide fraction with broad antibacterial activity. *Asian Journal of Biochemistry*, 4(3): 77-87.

Chauhan, A. & Mittu, B. (2015). Phyto-chemical screening and anti listerial activity of Annona Muricata (L) leaf extract. *Journal of Chromatography & Separation Techniques*, 6(3): 269.

Clinical and Laboratory Standards Institute (CLSI) (2012). *Performance standards for antimicrobial disk susceptibility tests*; Approved standard – Eleventh edition. CLSI document M02-A11. Wayne, PA: Clinical and Laboratory Standards Institute.

Cowan, M.M. (1999). Plant products as antimicrobial agents. *Clinical Microbiology Reviews*, 12(4): 564-582.

Francis, R. & Keinath, A. (2010). Biofungicides and Chemicals for Managing Diseases *In* Organic Vegetable Production. CLEMSON Cooperative Extencion Information Leaflet. Pp. 88.

George, V.C., Kumar, D.N., Suresh, P.K. & Kumar, R.A. (2015). Antioxidant, DNA protective efficacy and HPLC analysis of Annona muricata (soursop) extracts. *Journal of Food Science and Technology*, 52(4): 2328-2335.

Hossain, F., Ali, O., D'Souza, U.J. & Naing, D.K.S. (2010). Effects of pesticide use on semen quality among farmers in rural areas of Sabah, Malaysia. *Journal of Occupational Health*, 52(6): 353-360.

Nuttaporn, S. (2007). *Isolation and identification of galangin and other compounds from* Alpinia galanga *linnaeus willd and* Alpinia officinarum *hance* (MSc thesis), Suranaree University of Technology.

Peraica, M., Radic, B., Lucic, A. & Pavlovic, M. (1999). Toxic effects of mycotoxins in humans. *Bulletin of the World Health Organization*, 77(9): 754-766.

Pereira, A.P., Ferreira, I.C., Marcelino, F., Valentão, P., Andrade, P.B., Seabra, R., Estevinho, L., Bento, A. & Pereira, J.A. (2007). Phenolic compounds and antimicrobial activity of olive (*Olea europaea* L. Cv. Cobrançosa) leaves. *Molecules*, 12(5): 1153-1162.

Qader, S.W., Abdulla, M.A., Chua, L.S. & Hamdan, S. (2012). Potential bioactive property of *Polygonum minus* Huds (kesum) review. *Scientific Research and Essays*, 7(2): 90-93.

Seo, J.W., Cho, S.C., Park, S.J., Lee, E.J., Lee, J.H., Han, S.S., Pyo, B.S., Park, D.H. & Kim, B.H. (2013). 1′-Acetoxychavicol acetate isolated from *Alpinia galangal* ameliorates ovalbumin-induced asthma in mice. *PLoS One*, 8(2): e56447.

Thitilertdecha, N., Teerawutgulrag, A. & Rakariyatham, N. (2008). Antioxidant and antibacterial activities of *Nephelium lappaceum* L. extracts. *LWT-Food Science and Technology*, 41(10): 2029-2035.

Wightwick, A., Walters, R., Allinson, G., Reichman, S. & Menzies, N. (2010). Environmental risks of fungicides used in horticultural production systems. *In* Carisse O. (Ed), *Fungicides*, InTech. Pp. 273-304.

SHORT COMMUNICATION

The Geology of Upper Baleh River, Kapit, Sarawak

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ABSTRACT

Geological mapping of the proposed Baleh National Park, Sarawak was conducted during the Heart of Borneo Expedition in Mid November 2015 with Institute of Biodiversity and Environmental Conservation, Universiti Malaysia Sarawak. A geological map of the study area is compiled together with maps of the previous studies. The proposed Baleh National Park is made up of plateau and mountain chains. The topography of the study area is closely related to the geology. The plateau is underlain by the volcanic rocks which consists predominantly of tuff and dacitic rocks with scattered agglomerate, while the mountain chains are the ridges which striking east-west direction are underlain by slate interbedded with siltstone, sandstone and mud clast conglomerate of the Layar Member. The Layar Member of the Belaga Formation is suit of deep ocean marine deposits during the Late Cretaceous [100.5–66 million years ago (ma)]. The plateau of the Bukit Tiban was formed as a result of the volcanic eruption during the Late Miocene (11.6–5.3 ma). Several interesting geoheritage sites were observed in the study area.

Keywords: Bukit Tiban, columnar joints, dacite, geoheritage, Layar Member

The recent geological mapping of the Bukit Batu Tiban area is part of the proposed Baleh National Park. The site visit was carried out within a span of 11 days from 19th November until 29th November 2015. A group of 30 scientists from various background on flora and fauna travelled to collect baseline data before the area are to be gazetted as National Park. The distribution of rocks is significant in the study of flora and fauna diversity. The nature of the bedrocks fundamentally influences its overlying soil, and many plants and animal species are typical of certain soils that in turn are associated with certain underlying rocks. The objectives of the study were to map the geological formation which underlain the proposed Upper Baleh National Park as well as to locate the potential geoheritage sites.

The study area is located east of Kapit Town. It is bound by Longitude 114^0 6' E to 114^0 36' E and Latitude 1^0 25' N to 1^0 35.6'N (Figure 1). The overall accessibility to the study area is very poor. The area is connected by the logging track which is only accessible to the major logging pond at Putai. Putai is well served by the express boat once daily.

The geological mapping was carried out along designated route with different lithological characters to confirm the studies by the previous researchers. The access road from Logging base camp to Bukit Tiban and selected rivers were traversed using Global Positioning Station (GPS) to make accurate geological map. Rock samples were collected and geometrical aspect of outcrop were studied stratigraphically and structurally.

Topography – The topography of the area is made up of plateau and the mountain chains. The plateau area which is about 1000 m above sea level are located at the most eastern part of the study area bordering the Kalimantan, Indonesia. While the tableland stands above 1000 m, the area below is about 300 m to 1000 m above sea level formed mountain chains. The topography of the area is closely related to the geology. The tableland is underlain by the volcanic rocks which consists predominantly of tuff and dacitic rocks with scattered agglomerate. The prominent topographical features of these volcanic rock-type are very steep cliffs. The topography of the areas below the tableland is totally different; the mountain chains are the ridges which striking east-west direction are underlain by sedimentary
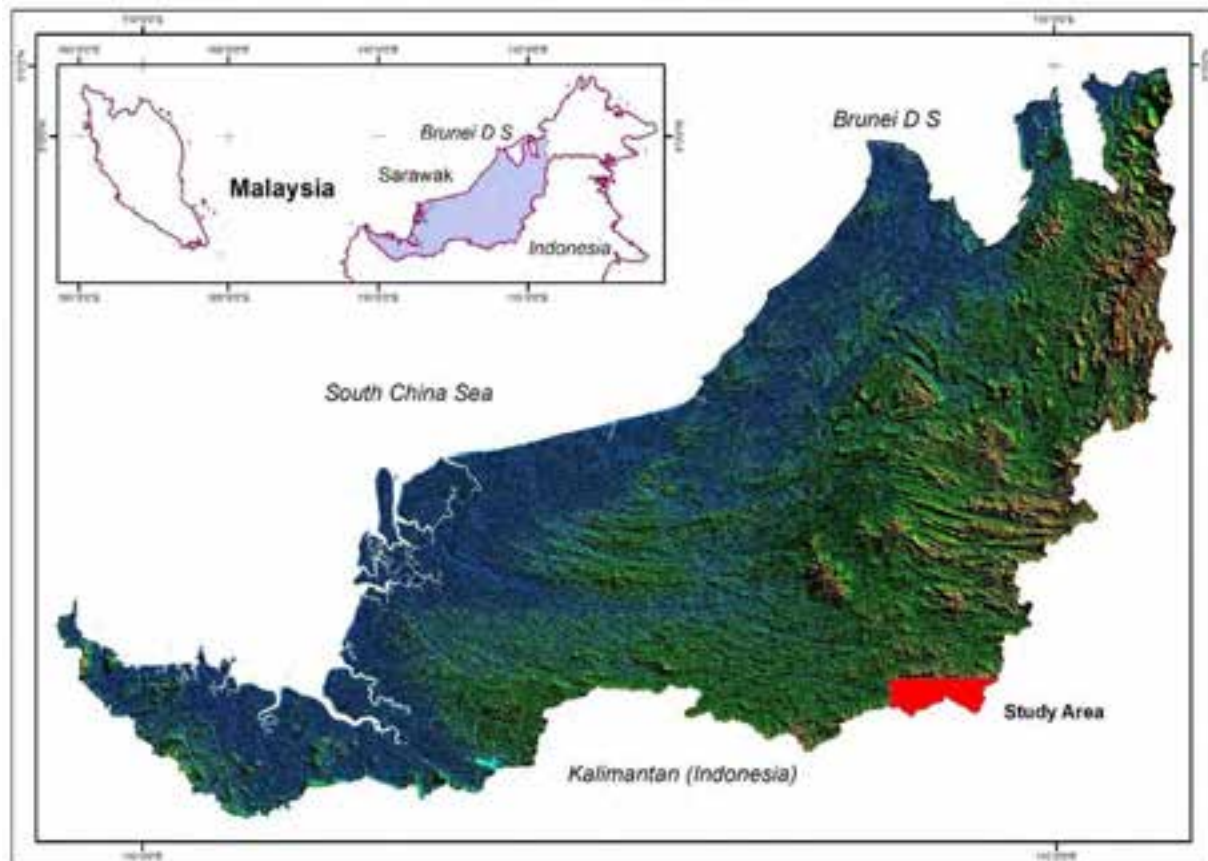
Figure 1. The study area is located at the eastern part of Sarawak bordering with Kalimantan, Indonesia.

rock (Figure 2). These ridges were formed when the sedimentary rock formation were folded.

Drainage – Batang Baleh formed the main drainage system in the study area. The rivers and streams make up generally trellis to dendritic drainage patterns in the study area. The overall drainage pattern of the study area which is underlain mostly by arenaceous rocks of the Layar Member is of a trellis type. The drainage pattern is due to the folded strata of the interbedded sandstone–shale which dominated the eastern part of the study area. It is also controlled by the joint and fault system that are intersecting perpendicularly to the east-west trending beds.

The eastern part of the study area which is underlain by igneous rocks of extrusive type exhibits drainage patterns of dendritic (Figure 3). The drainage pattern is controlled by the geological structure and the resistance of the volcanic rock which also shaped the topographical features.

Geological settings – The geology of Sarawak can be subdivided into three distinct provinces which correspond to three main geographic regions, namely West Sarawak, Central Sarawak and North Sarawak (Figure 4). The geology of West Sarawak is characterised by extensive areas of Triassic sediments, intrusives, metamorphics and volcanics (Banda, 1992). These are overlain in some areas by Late Jurassic to Eocene sediments, which are in places intruded by Late Cretaceous igneous rocks. The Lupar Line separates West Sarawak from Central Sarawak and has been interpreted as a subduction zone (e.g. Hutchison, 2005) or as a major strike-slip fault (e.g. Haile *et al*., 1994). Central Sarawak is dominated by Upper Cretaceous Rajang Group of turbidites, which have a deep water, probably distal character and are intensely folded, faulted and thrusted. These have been interpreted as an accretionary prism (e.g. Hutchison, 2005). North Sarawak is the region north and east of the Rajang-Baram Watershed. This region is underlain by Neogene sediments
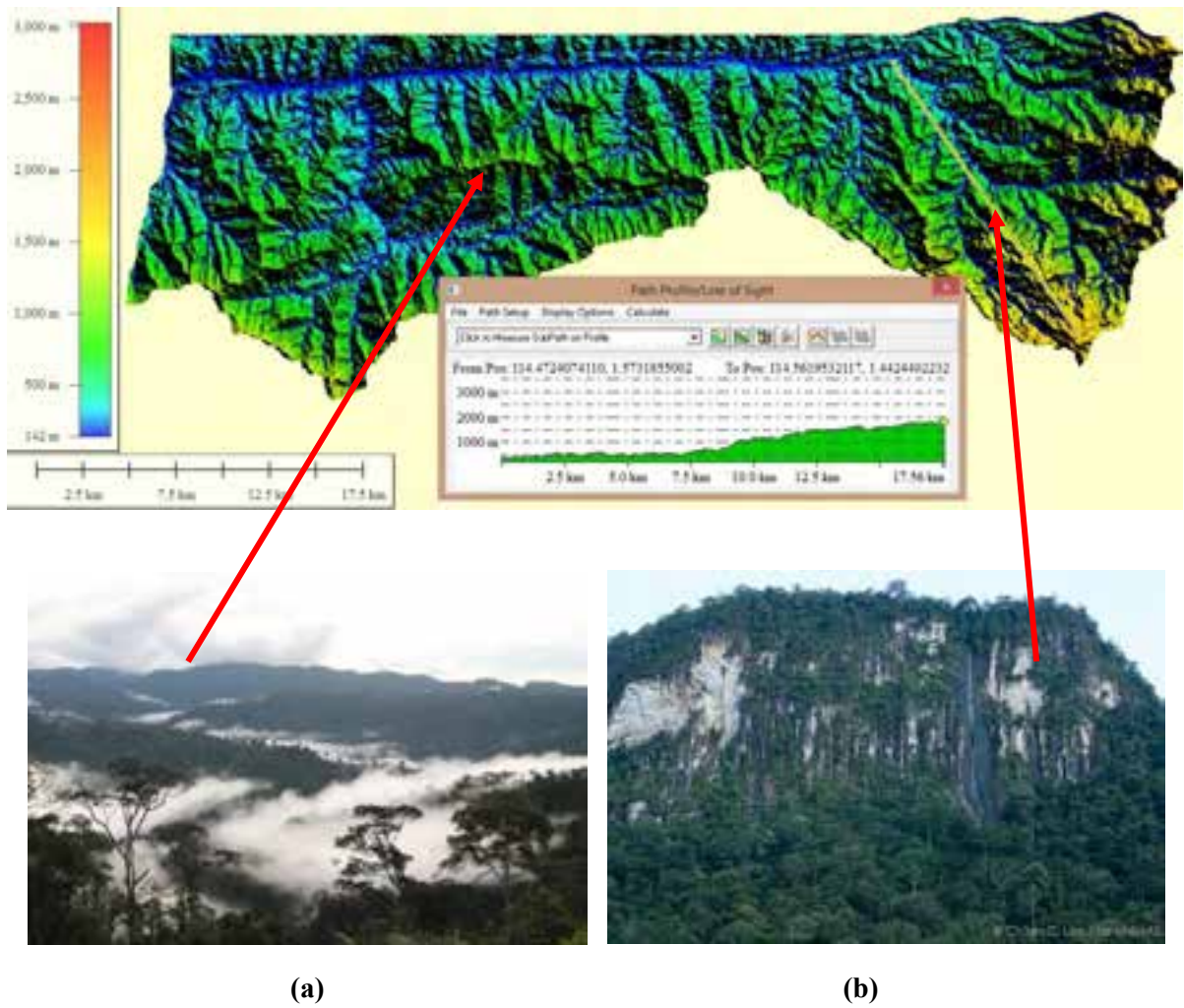
(a)   **(b)**

Figure 2. On top shows the topographical map of the study area with the view of two different topographical features. (a) Ridges striking east-west direction are underlain by sedimentary rock; (b) Steep cliffs of volcanic rock.
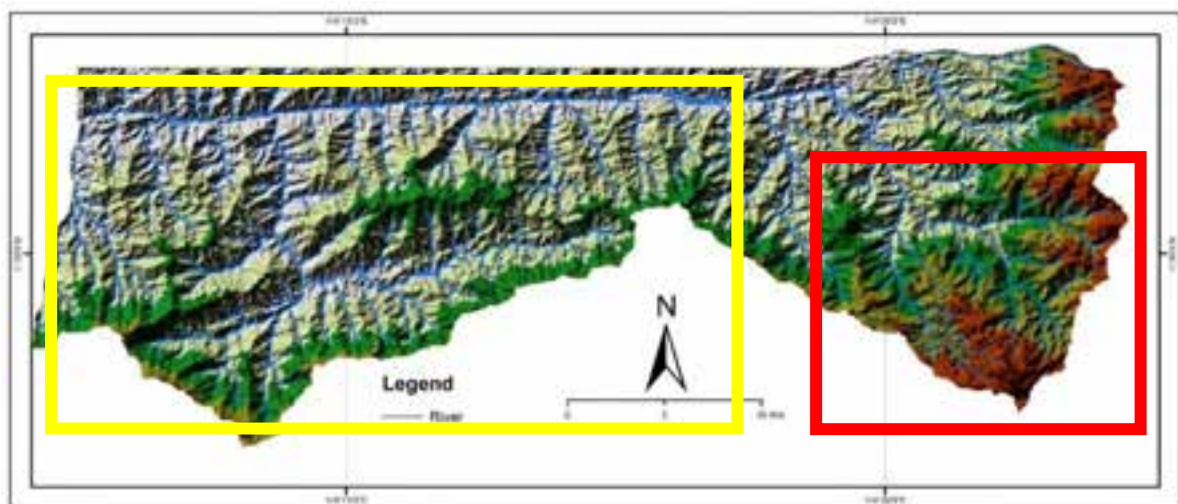

Figure 3. The drainage map which shows the trellis pattern (yellow box) and dendritic pattern (red box).
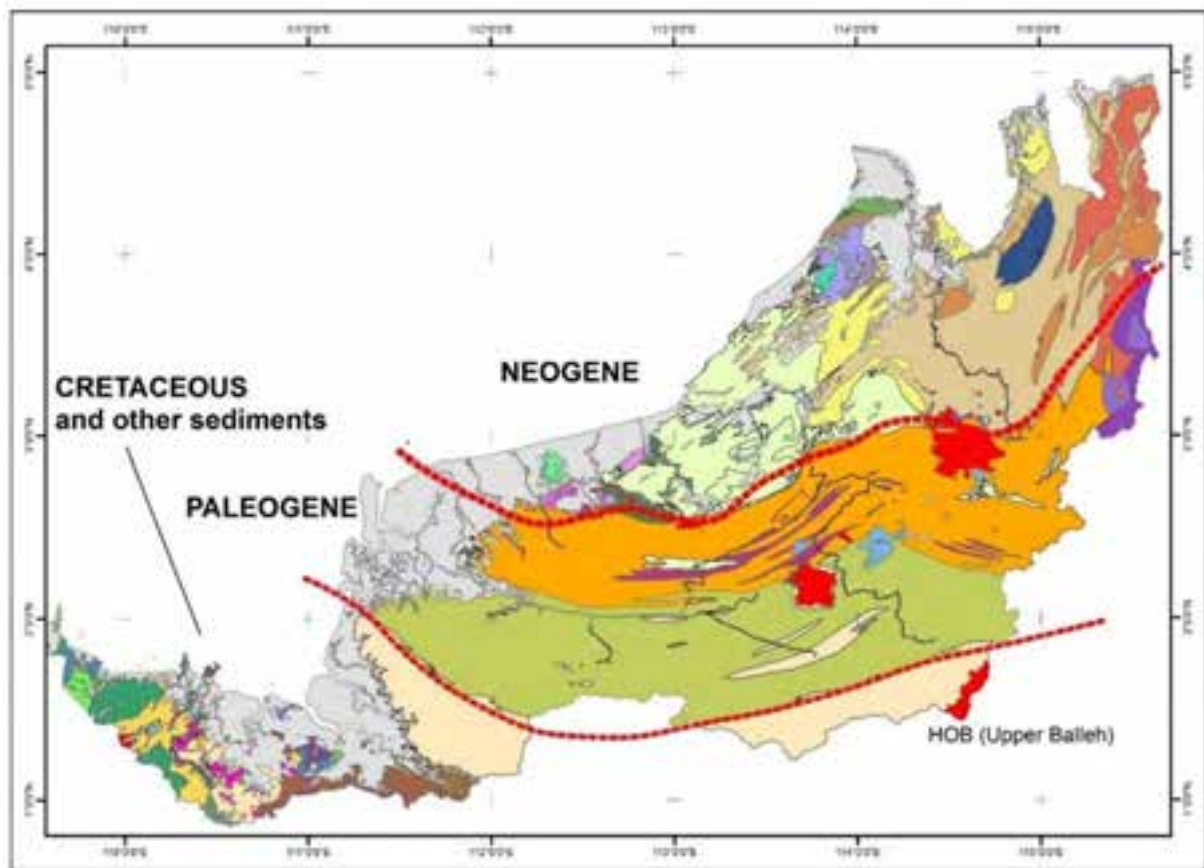
Figure 4. The geological setting of Sarawak are subdivided into three distinct provinces (after Banda, 1992).

of the NW Borneo Basin. Two phases of sedimentation occured during the Neogene Period which resulted in the deposition of thick marine argillaceous sediments overlain by the shallow-water sediments consisting of arenaceous coarse clastics (Banda, 1992).

Regional geology – The study area is located in the area dominated by the Cretaceous and Eocene to Miocene Crocker-Rajang-Embaluh accretionary complex in Central Sarawak. It consists primarily of turbidites which were being shed northeastward off the Schwaner and younger volcanic arcs into a paralic to deep marine trench basin. These sediments were imbricated, deformed, and weakly metamorphosed during the Cretaceous subduction and finally were intruded by late stage and post subduction intrusions of the Sarawak Orogeny. The plateau of Bukit Batu Tiban or generally term as Neuwenhuis

Mountains or Mentulang Plateau in Kalimantan occurred during the volcanic activities in Miocene-Pliocene (Banda, 1992).

Site geology – The geological stratigraphy of the Bukit Batu Tiban area is summarized in table 1. The oldest known rock unit is the Layar member of the Belaga Formation (Late Cretaceous) and were deposited in a deep marine. The member consists of mudstone-dominated facies as the lower unit, which coarsens upwards into siltstone-sandstone-conglomerate dominated facies. The base of the Layar Member is in Lubok Antu where it is fault contact with the Lupar Formation (Liechti *et al.*, 1960). The overlying stratigraphic and the youngest unit is the Bukit Batu Tiban Volcanics. This unit forms the highest and sometimes lookalike karst topography. It is made up mainly of dacite, tuff, basalt and angglomerate.

Table 1. General stratigraphy of the study area.

Regional Event	Lithostratigraphic Unit	Age (Million years, ma)
Volcanic eruption in Central Sarawak	Batu Tiban Volcanic/ Mentulang Volcanic	Late Miocene (11 ma)
Late Eocene folding		
In Central Sarawak	Layar Member, Belaga Formation	Eocene (56 ma)
Deposition of turbiditic sandstone and shale		

Layar Member (Belaga Formation) – The Layar Member of the Belaga Formation (Late Cretaceous) is part of the Rajang Group which also include the Lupar Formation and other member of the Belaga Formation. In Kalimantan, the Rajang Group extend into Embaluh and Selangkai Formation. The Layar Member which is generally composed of predominantly of slate and phyllite with rhythmically interbedded metagraywacke (Tan *et al*., 1980) are found at the eastern part of the study area. Based on the field mapping by Banda (1992), the Layar Member are divided into four (4) facies, namely; i) Mudstone-dominated facies, ii) Siltstone-dominated facies, iii) Sandstone-dominated facies and, iv) Conglomerate-dominated facies.

During the recent field trip, few rivers and roads were traversed to located and confirm the dominated facies as described by Banda (1992). Among the traversed route are as shown in Figure 5. Mudstone-dominated facies consists of mudstone interbedded with siltstone forms the lower part of the sections. Amongst the sedimentary structures found in this facies are parallel-continous bedding, graded bedding, load structures, small scale cross-bedding and slumping. Siltstone-dominated facies comprises of siltstone interbedded with mudstone (Figure 6). These beds are similiar to the mudstone-dominated facies except that the siltstone beds are the dominant lithology and are generally thicker.
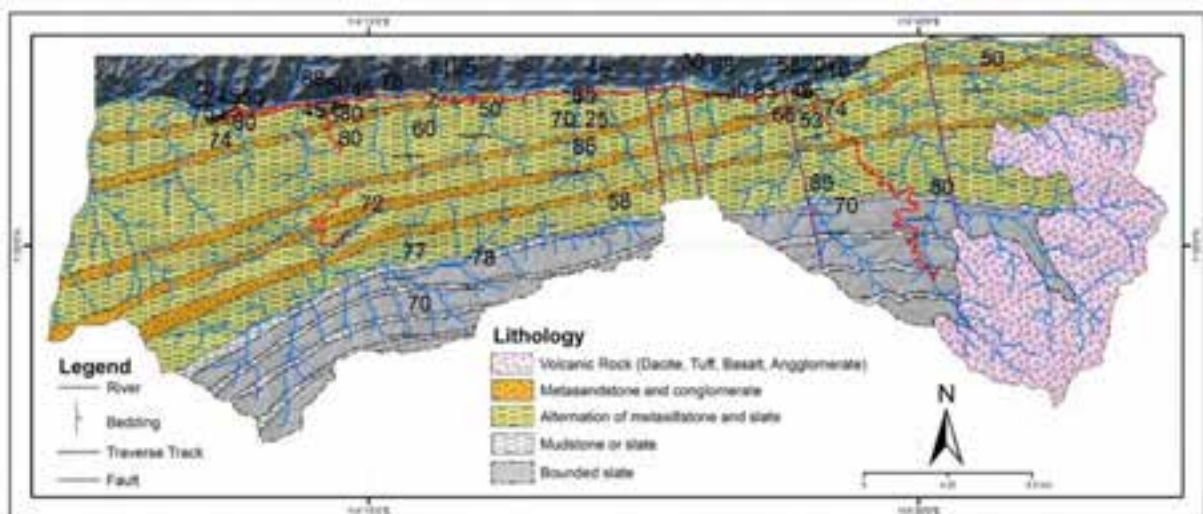

Figure 5. The traversed route of the study area.

Sandstone-dominated facies consists of fine to coarse-grained sandstone interbedded with siltstone and a little mudstone. The massive sandstone is of 30 cm to 3 m thick with sedimentary structures such as graded bedding, flute cast and channelling are commonly found at the base of the sandstone beds (Figure 7).

Conglomerate-dominated facies which forms the top unit of the Layar Member consists of coarse-grained sandstone interbedded with conglomerate. Two types of conglomerate were observed along the Sungai Grugu are para and ortho-conglomerate. Para-conglomerate consists of rafted slabs of mudstone in a coarse-grained matrix and ortho-conglomerate consists of sub-rounded to rounded pebbles of quartz, sandstone and chert embedded in a matrix of coarse-grained sandstone (Figure 8).

The coarsening upwards sequence commonly observed in submarine deltas is the result of mass movement and at the same time, channelling along the delta slope. The conglomerate and sandstone-dominated facies deposits are interpreted as mass-flow products and the mudstone-dominated facies as turbidites deposited in a quiet environment.

Igneous Rock – The igneous rock in the study area comprises of extrusive. This youngest unit of rocks is overlying the Layar Member of the Belaga Formation consists of extrusive igneous such as dacite, tuff and agglomerate. The tuff forms the tableland whereas the dacite forms isolated hill with karst-like features (Figure 9). Dacite is found along the Sungai Menuang Ili as outcrop and big boulder (Figure 10; Banda, 199). Fresh dacite hand specimen is light grey in colour, porphyritic with coarse-grained phenocrysts of feldspar, quartz, biotite in a fine groundmass (Figure 11).

The tuff contains rock fragments embedded in the feldspathic matrix and it is extensive in the study area overlying the dacitic rock forming the plateau. Agglomerate is very minor and associated with tuff with patches. The agglomerate occurs as pyroclastic flow, consisting mainly of rock fragments embedded within welded tuff. The fragments are of several sizes from 1 mm to a few centimetre

across. It is commonly observed in the Sg. Menuang Ili as boulder.

Figure 6. Slate with thin beds of metasiltstone.

Figure 7. Thick metasandstone beds which dominate the upper layer of Layar Member.

Figure 8. The grab sample of conglomerate with rounded clast of sandstone, slate and quartz.

Figure 9. Karst-like features of dacitic cliff with waterfalls.

Figure 10. Boulders of dacitic rocks found along the Sg. Menuang Ulu.
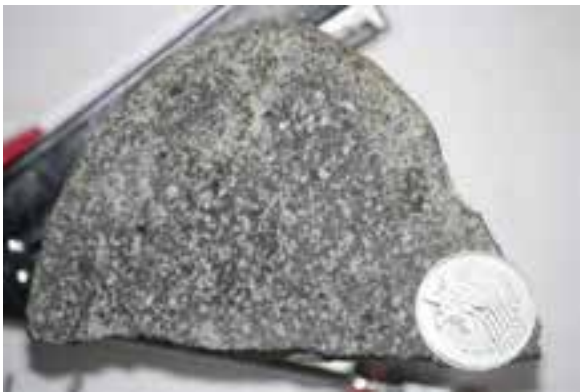

Figure 11. Hand specimen of fine grained, light grey dacitic rock.

The intrusive rocks are very minor, scattered in a few places in Sg. Menuang and along the access timber track. They occurred as basaltic sill and dykes, intruded into the Layar member. The basalt dykes which occur in the form of columnar jointing body are found along the access logging track (Figure 12a).

Geoheritage – According to Brocx and Semeniuk (2007), geoheritage (geological heritage) is a concept concerned with the preservation of features with importance to earth science, such as landforms, natural and artificial exposures of rocks, and sites where geological features can be examined. Geoheritage is a descriptive term applied to sites or areas of geologic features with significant scientific educational, cultural or aesthetic value (Geological Sociey of America, 2011). Scientifically and educational significant geoheritage sites include those with textbook geological features and landscapes, distinctive rock or mineral types, unique or unusual fossil or other geological characteristic. Cultural significant geoheritage – geological features or landscape played a role in cultural or historical events. Aesthetic significant geoheritage – landscape visually appealing – geological features or processes.

Ulu Baleh possesses rich geodiversity in terms of rocks, geological structures, geomorphological and landscape features. Geoheritage of Ulu Baleh mostly occurred within rocky riverbanks, cliffs, peaks and waterfalls. Some highly significant exposures are potentially to be classified as geoheritage sites, containing one or more geodiversities of high heritage value. Some of the geoheritage sites identified are the basaltic columnar joints (Figure 12a) and waterfall with metasediment outcrops (Figure 12b) which possessed significant value, whereas another geoheritage sites with aesthetic value are dacitic rock cliff

Figure 12. Some outstanding geological features with scientifically and educational value. (a) Basaltic dyke which occurred at columnar joints; (b) Outcrop of metasediment with waterfall.

Figure 13. Some outstanding geological features with aesthetic value; (a) Dacitic cliffs with "karst" like features; (b) Colluvium deposits along Baleh river

and colluvium deposits along the Baleh river (Figure 13). The outcrops of basalt and metasediment are significant to education and research as these features are the evidences of the geological process that occurred in the study area millions years ago. Aesthetically significant geoheritage sites of cliffs of dacitic rocks with very high waterfalls and colluvium deposits that are visually appealing because of their geologic features or processes can be tourist destinations and provide local and regional economic benefits.

The upper reaches of the Baleh river are still rich in nature, especially the natural beauty of its topographical features formed by geological processes. The geological formation which age ranges from eocene to late miocene consist of regionally metamorphosed sediments of slate, metasiltstone, metasandstone and mud clast conglomerate. These metasediments are overlain by dacitic, tuff and agglomerate of tertiary volcanic, and intruded by basalt sill. The geomorphological and geological features shape the areas as the potential geoheritage that needs to be preserved such as basaltic columnar joints, karst-like features of dacitic cliff, colluvium along riverbanks and waterfalls at the dacitic cliff.

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REFERENCES

Banda, R. M. (1990). The geology and planktic foraminiferal biostratigraphy of the northwest Borneo Basin, Sarawak, Malaysia. Thesis of PhD, University Tsukuba, Japan.

Banda, R.M. (1992). Progress Report: Geological Mapping, Bukit Batu Tiban Area. Proceeding of the 23rd Annual Geological Conference 1992, Technical Papers, Pp 118-125.

Brocx, M., & Semeniuk, V. (2007). Geoheritage and geoconservation: history, definition, scope and scale. *Journal of the Royal Society of Western Australia*, 90: 53-87

Geological Society of America. (2011). Geoheritage. http://www.geosociety.org. Downloaded 1 December 2018.

Haile, N.S., Lam, S.K., & Banda, R.M. (1994). Relationship of gabbro and pillow lavas in the Lupar Formation, West Sarawak; implications for interpretation for the Lubok Antu Melange and the Lupar Line. *Bulletin of the Geological Society of Malaysia*, 36: 1-9.

Hutchison, C.S. (2005). *Geology of North West Borneo, Sarawak, Brunei and Sabah*. UK: Elsevier, Pp 67-80.

Liechti, P., Roe, F.W., & Haile N.S. (1960). *The Geology of Sarawak, Brunei and the western part of North Borneo*, British Territories of Borneo, Geological Survey Department, Bulletin no. 3, Pp 360.

Tan, D. N. K., Kho, C. H., & Hon. V. (1980). *Regional geology, Sarawak*. Geological Survey Malaysia Annual Report 1979, Pp 97-118.