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Diversity of Phytotelmic Mosquito is Not Correlated with the Diversity of Phytotelmata: A Survey Report from Lampung, Indonesia

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Abstract: Statistical records released by Lampung provincial health officials that incidence of dengue hemorrhagic fever (DHF) mostly occur in January to February each year, the question is what factors and conditions contribute to such fenomenon? Current work intended to investigate whether the DHF incidence relates to the seasonal diversity of phytotelmata and mosquito larvae inhabit such small ponds. Three neighboring districts, ranked the top three for the DHF annual incident rate in Lampung province, were included in the study. The survey was carried out on February, March, May and June 2016. February is the peak of rainy season in which DHF incidence was at the highest level, while June is the beginning of dry season. The criteria of phytotelmata used in the study were every structures present in plants which allow rain water to impound which visually showed the signs of life in it. Phytotelmata found to contain larvae of Aedes mosquitoes then prepared for further analysis and the data are presented descriptively. There were 25 plant species found to presenting parts which fulfilled the criteria of phtytotelmata, and seven types of phytotelmata were identified namely leaf axils, tree holes, tree stumps, fallen spathe, open fruit shells, fallen leaves, and flower spathes. There are ten species of mosquito larvae found, with *Aedes albopictus* is predominant. The Shannon-Wiener diversity index of the phytotelmata is lowest in June (1.50) compared with that of in February (2.13), March (2.07) or May (2,31). Interestingly, the diversity index of mosquito larvae is highest in June (1.18) and lowest in March (0.71). Statistically, both phytotelmata diversity and mosquito diversity are not correlated with physical factors. In addition, the Shannon-Wiener diversity index of mosquito larvae it self is not correlated with the diversity index of phytotelmata. It is suggested that the mosquito diversity is not related to the divesity of phytotelmata.
Keywords: phytotelmata, Aedes mosquito, dengue hemorrhagic fever, vector mosquito, DHF vector control

INTRODUCTION

Provincial health statistics of Lampung province of Indonesia released by the provincial health officials revealed that the incidence of dengue hemorrhagic fever (DHF) occurs mostly in January to February every year. In the period of 2000 -2016, in the province with a population of about 8 million, more than 20,000 people suffered from DHF and more than 150 of them died [1,2]. Indonesia's health authorities, including the Lampung provincial health office, have made DHF a priority in health development programs, including by incorporating the disease-prevention program into the primary and secondary schools curricula. As a result, today most of Indonesians have knowledge of the symptoms, causes, and strategies for vector eradication of this disease [3].

With regard to the vector control, particularly against mosquitoes, most people in the country know that breeding places of the DHF disease vectors include ponds, water container, or a variety of man-made waste items such as discarded tyres or used cans. However there are too few people aware that such small water bodies found in or upon plants, which were called by Ludwig Varga in 1928 as phytotelmata [6], should also be taken into account. In fact, various species of vector mosquitoes such as Aedes sp. were found to breed in such hanging small ponds including tree holes, leaf axils, bamboo joints, or papaya stumps [4,5].

In Indonesia, study on phytotelmata has not been done in earnest [7]. However, in our previous works, we reported that in Lampung phytotelmata is presumed to have a role in the annual incidence of DHF [8]. Considering statistical records released by Lampung provincial health officials that incidence of DHF mostly occur in January to February each year, the question is what factors and conditions contribute to such fenomenon? Current work intended to investigate whether the DHF incidence relates to the seasonal diversity of phytotelmata and mosquito larvae inhabit such small ponds.

METHOD

Study Area

Three neighboring districts, ranked the top three for the DHF annual incident rate in Lampung province, were included in the study namely Pesawaran, Pringsewu and Bandar Lampung. The survey was carried out on February, March, May and June 2016. February is the peak of rainy season in which DHF incidence was at the highest level, while June is the beginning of dry season[9].

Inclusion Criteria

The phytotelmic criteria used in the study were adopted from Kicthing [6] i.e. every structures present in terrestrial plants such as modified leaves, leaf axils, flowers, stem holes or depressions, tree stumps, open fruits and fallen leaves, which allow rain water to impound in which the signs of life was seen visually. Plants that were observed include wild and crop plants that grown naturally or cultivated both in backyard and plantation areas.

Sampling

The sampling procedure undertaken in this study is similar to the procedure we applied in the previous survey [8]. After the plant was photographed for taxonomic identification, the impounded water in each phytotelmata was collected using a suction pump. The phytotelmic fluid then placed separately in labeled vials and transported to the laboratory for further analysis. At the laboratory, the mosquito larvae were sorted and separated from trash and other debris. The dead larvae were separated from the living larvae and put into bottles containing 70% alcohol for toxonomic identifiaction.

Data Analysis

The diversity of phytotelmata and mosquito larvae in the survey months was estimated using Shannon-Wiener's Index (H'). For the *post hoc* analysis, the diversity index between months are compared using Student's t-Test at $P < 0.05$ [10]. To assess association between variables a linear regression-correlation statistics was used.

RESULTS

Phytotelmic Plants

Plant species found to contain structures which allow rain water to impound in which the signs of life can be seen visually obtained in this survey are listed in Table 1.

Table-1: Plant species and their parts that impound water and functioned as phytotelmata found in the survey

Plant species	Phytotelmic parts						
	Leaf axils	Tree holes	Tree stumps	Fallen spathe	Fruit shells	Fallen leaf	Flower Spathes
Alocasia macrorrhiza	√						
Ananas comosus	√						
Arenga pinnata				√			
Canna indica	√						
Carica papaya			√				
Cocos nucifera		√		√	√		
Coffea robusta		√					
Colocasia esculenta	√						
Dracaena fragrans	√						
Curcuma domestica							√
Gigantochloa apus			√				
Gigantochloa atroviolacea			√				
Guzmania sanguinea	√						
Hevea brailiensis		√					
Leucaena leucocephala		√					
Mangifera indica		√					
Musa paradisiaca	√			√		√	
Nephelium lappaceum		√					
Pandanus amaryllifolius	√						
Pyracantha angustifolia		√					
Roystonea regia				√			
Saccharum officinarum	√						
Sanseviera trifasciata	√						
Senna siamea		√					
Theobroma cacao		√			√		
Total	10	9	3	4	2	1	1

On the 25 phytotelmic plant species, there are seven types of the structures revealed to contain water in which mosquito larvae are found namely leaf axils, tree holes, tree stumps, fallen spathe, fruit shells, fallen leaves, and flower sapathes. Among those six structures, leaf axils (n=10) and tree holes (n=9) are the most plant parts commonly function as phytotelmata. Fallen leaves and flower spathes, though potentially flooded, but very rarely function as phytotelmata. In this study, only one fallen leaf (of *Musa paradisiaca*) and one flower spathe (of *Curcuma domestica*) found to function as phytotelmata.

Phytotelmata and the Inhabitants

The number of types and individuals of phytotelmata in each survey month are presented in Table 2**.** Based on the data in Table 2 it seems that not always an impounded water in certain part of the plant serves as a phytotelma.

Table-2: Number of types and individuals of phytotelmata found in each survey month

Plant species and its phytotelmic parts	February	March	May	June
Alocasia macrorrhiza (leaf axils)	1	28		
Ananas comosus (leaf axils)	2	4	3	2
Arenga pinnata (fallen spathe)			1	
Canna indica (leaf axils)			2	
Carica papaya (tree stump)			1	
Cocos nucifera (fallen spathe)	8		2	
Cocos nucifera (fruit shells)		10		
Cocos nucifera (tree hole)			6	3
Coffea robusta (tree hole)			10	4
Colocasia esculenta (leaf axils)	6	15	19	11
Curcuma domestica (leaf axils)			1	
Dracaena fragrans (leaf axils)	6	24	13	
Gigantochloa apus (tree stump)	13	35	1	1
Gigantochloa atroviolacea (tree stump)		31		
Guzmania sanguinea (leaf axils)	4			
Hevea brailiensis (tree hole)			1	1
Leucaena leucocephala (tree hole)		1		
Mangifera indica (tree hole)		1		
Musa paradisiaca (fallen leaf)		1		
Musa paradisiaca (fallen spathe)			8	
Musa paradisiaca (leaf axils)	18	65	20	15
Nephelium lappaceum (tree hole)	1			
Pandanus amaryllifolius (leaf axils)	1	12		
Pyracantha angustifolia (tree hole)	3			
Roystonea regia (fallen spathe)			1	
Saccharum officinarum (leaf axils)				
Sanseviera trifasciata (leaf axils)				
Senna siamea (tree hole)		1		
Theobroma cacao (tree hole)	5	3	14	33
Number of Types (S)	12	14	16	8
Number of Individuals (N)	68	230	104	70

Table-3: presented the number of types and individuals of mosquito larvae found to inhabit phytotelmata in each survey month. The data clearly show that among of ten mosquito larvae species, *Ae. albopictus* is predominant.

Shannon-Wiener's diversity index of phytotelmata and mosquito larvae obtained in each survey months as well as the results of *post hoc* test are presented in Table 4.

The data revealed that the diversity of phytotelmata as well as mosquito larvae is differed between months. Phytotelmata diversity is highest in May and lowest in June, in contrast the diversity of mosquito larvae is highest in June and lowest in March.

Table-3: Number of types and individuals of mosquito larvae collected from phytotelmata in each survey month

Mosquito Larvae	February	March	May	June
Ae. albopictus	142	321	363	81
Ae. aegypti	0	0	50	5
Ae. chrysolineatus	24	51	8	20
Cx.quenquefasciatus	25	49	47	14
Cx.fragilis	0	0	19	0
Cx. lophoceraomiya	0	0	1	0
Armigeres sp.	0	0	4	3
Tripteroides sp.	0	0	24	0
Toxorhyncites sp.	1	0	0	1
Anopheles sp.	0	0	1	4
Number of Species (S)	4	3	9	7
Number of Individulas (N)	192	421	517	128

Table-4: Comparison of the diversity index of phytotelmata and mosquito larve between survey months

Variables	Diversity index (H')			
	February	March	May	June
Phytotelmata	2,13[ab]	2,07[a]	2,31[b]	1,50[c]
Mosquito larvae	0,78[a]	0,71[a]	1,08[b]	1,18[b]
H' values in the same row followed by different superscript are statistically different (*P* > 0.05) based on t-test				

Physical Factors

Total rainfall, phytotelmic water temperature, and phytotelmic water pH in averages in each survey month are presented in Table 5. In this study period, rainfall gradually decreases to reach the lowest level in June (from 305 mm to 96 mm) . In contrast, pH increases gradually during this period (from 6 – 7.1).

Table-5: Average of physical factors of the phytotelmata in each survey month

Variables	February	March	May	June
Total rainfall (mm)	305	217	128	96
Phytotelmic water temperature (°C)	25,7	25,2	25	26,8
Phytotelmic water pH	6	6	6,4	7,1

Table 6, 7 and 8 are consecutively presenting results of regression-correlation analysis between diversity of phytotelmata and physical factors, diversity of mosquito larvae and physical factors, and between diversity of mosquito larvae and diversity of phytotelmata. Regarding Table 8, it can be ascertained that the diversity index of mosquito larvae is not only unpredictable but also totally not correlated to the diversity index of phytotelmata.

Table-6: Correlationship between diversity of phytotelmata and physical factors

Physical Factors (Independent variable)	Phytotelmata (Dependent Variable)			
	R	R²	F	P
Precipitation	0,456	0,208	0,526	0,544
Temp	-0,938	0,879	14,546	0,062
pH	-0,783	0,613	3,174	0,217
R=coefficent of correlation; R²=coefficent of determination; F=Anova; P=level of probabilty				

Table-7: Correlationship between diversity of mosquito species and physical factors

Physical Factors (Independent variable)	Mosquito (Dependent Variable)			
	R	R²	F	P
Precipitation	-0,768	0,589	2,868	0,232
Temp	0,066	0,004	0,009	0,934
pH	0,628	0,394	1,302	0,372
R=coefficent of correlation; R²=coefficent of determination; F=Anova; P=level of probabilty				

Table-8: Correlation between diversity of mosquito larvae and diversity of phytotelmata

	Mosquito (Dependent Variable)			
Phytotelmata (Independent variable)	R	R^2	*F*	*P*
	-0,008	0,000	0,000	0,992
R=coefficent of correlation; R^2=coefficent of determination; F=Anova; P=level of probabilty				

DISCUSSION

Among 25 species of phytotelmic plants only three species namely coconut (*Cocos nucifera*), banana (*Musa paradisiaca*), and cocoa (*Theobroma cacao*) found to contain more than one part that serve as phytotelma, whereas the 22 species of the rests are only provide one (Table 1). Among the three species, banana is the most plant provide phytotelmata in almost all survey months (Table 2). This allegedly due leaf axils of banana are producing dew that contribute to the water in the phytotelmic axils. In addition, the structure of the leaf base in some varieties reduces evaporation[11].

As has been mentioned in introduction section, the peak incidence of DHF in Lampung province occures in January to February each year. However, this study data show that, besides *Ae. albopictus, Ae. chrysolineatus, Cx.quinquefasciatus,* and *Toxorhynchites* spp., there was no *Aedes aegypti* larvae found in the period of February to March (Table 3). The question is why? Firstly, *Ae aegypti* mosquito prefers to breed indoor in a small water body such as in the open masonry tanks used for water storage, or out door in a small water body trapped in the discarded goods such as old tyres and cans. It may be that, during the wet season, small water bodies suitable for breeding place of Aedes aegypti are more diverse and dispersed [12,13].

Secondly, the DHF in the area surveyed is not only transmitted by the *Ae aegypti* mosquitoes but also, or mostly, by the *Ae albopictus*. It is very likely for the *Ae. albopictus*, commonly called as the Asian tiger mosquito, is known as a secondary vector of dengue[14]. In Indonesia alone, precisely in Banjarnegara, Central Java, the role of *Ae. albopictus* in dengue disease transmission is indicated by Rahayu and Ustiawan [15]. A strong evidence that *Ae albopictus* can contribute to the spread of dengue demonstrated by Rosa *et al*.[16]. By using RT-PCR methods for detecting dengue viruses on *Ae.albopictus* larvae inhabit phytotelmata they found two serotypes of dengue viruses DEN-1 and DEN-4 and suggested that the mosquito has the potential to transmit and spread DHF.

Thirdly, the *Ae.aegypti* larvae allegedly did not manage to compete with *Ae. albopictus* larvae. From malaysia it was reported that *Ae.aegypti* has lower competitive advantage compared to *Ae.albopictus* in situation of limited resources. This situation allegedly the cause of the dominancy of *Ae. albopictus* mosquito in Penang Island [16]. The high adaptability of *Ae albopictus* is illustrated by the extent of the spread of mosquitoes that originally indigenous to South-east Asia and islands of the Western Pacific and Indian Ocean, to Africa, the mid-east Europe and the Americas (north and south) [18,19].

Table 4 presents the summary of statistical results depicting diversity of phytotelmata as well as mosquito larvae between survey months. The diversity of phytotelmata is lowest in June (1.50) compared with that of in February (2.13), March (2.07) or May (2,31). Interestingly, the diversity index of mosquito larvae is highest in June (1.18) and lowest in March (0.71). The decline in phytotelmata diversity along with decreasing rainfall is logical. Such phenomenon not only occurs among mosquito but also in ciliate community, that the community changed substantially due to rainfall, however about 35% occured exlusively in dry season[20].

What physical factors account for the high diversity of mosquito larvae in June where rainfall is at the lowest level? Another physical factor known as a determinant factor for mosquito larvae to grow is temperature. From an experimental study it was reported, for example, that the optimum water temperature for the *Ae aegypti* mosquito larvae to grow is 30°C [21]. Therefore, it is logical that in this study *Ae aegypti* found only in May and June in which the average temperature of phytotelmic water reach 26.8 °C.

The data in Tables 6 and 7 clearly show that based on the correlation coefficient and the coefficient of determination, there are correlationship between physical factors and the diversity index of phytotelmata, as well between physical factors and the diversity index of mosquito larvae. However, analysis of variances of the correlation test yields an F-value and a P-value of which is more than 0.05. Thus it can be said that both the diversity index of fitotelmata and the diversity index of mosquito larvae can not be predicted by physical factors, especially rainfall, temperature, and pH. In this study, the diversity index of mosquito larvae it self even did not correlated with the diversity index of phytotelmata (Table 8). Overall, the range of physical factors in this study is still within the optimal range for the growth of mosquito larvae, especially for *Aedes albopictus* (Skuse), *Ae. aegypti* (Linnaeus), *Ae. vittatus*

(Bigot), *Anopheles* sp. *Culex quinquefasciatus* (Say) and *Toxorhynchites* sp[22].

CONCLUSION

The diversity index of phytotelmata is fluctuative but significantly decreased in June, the month with a lowest rainfall. In contrast, the diversity index of mosquito larvae collected from phytotelmata significantly higher in that month. However, during the survey periods, none of phytotelmata diversity nor mosquito larvae diversity predictable by physical factor changes. The diversity of mosquito larvae in question is not correlated with the diversity of phytotelmata.

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