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Single and co-breeding of different mosquito species in fogging-free and dengue risk areas in West Malaysia

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Abstract. Ovitrap surveillance was carried out in fifteen localities encompassing fogging-free and dengue risk areas in West Malaysia to determine on the dispersal and prevalence of single and co-breeding of mosquito larvae particularly *Aedes*. Ovitraps were sited randomly indoors and outdoors within human settlements in all study areas. All the localities exhibited positive ovitraps with single breeding of *Ae. albopictus* that ranged between 64.29% and 100.00%. These findings indicated *Ae. albopictus* as the predominant container-breeding species in all study areas. The co-breeding of *Ae. aegypti* with *Ae. albopictus* larvae (34 ovitraps), *Ae. albopictus* with *Culex quinquefasciatus* larvae (32 ovitraps) as well as *Cx. quinquefasciatus* with *Armigeres subalbatus* larvae (1 ovitrap) were also detected in certain study localities. Interestingly, co-breeding of *Ae. albopictus* with *Ar. subalbatus* larvae as well as *Ae. albopictus* with *Uranotaenia* sp. larvae in Malaysia is reported for the first time in the present study. Better understanding of the co-breeding scenario involving different species of mosquito larvae is needed to ensure the efficacy of vector control actions to be conducted.

INTRODUCTION

Mosquitoes continue to be a public health threat for their role in the spread of mosquito-borne diseases among human populations. *Aedes aegypti* and *Aedes albopictus* are primary vectors of dengue fever (DF), dengue haemorrhagic fever (DHF), yellow fever (YF), chikungunya and Zika virus worldwide (Moyes *et al.*, 2017). *Culex quinquefasciatus* plays an important role in transmitting lymphatic filariasis in several nations like Sri Lanka, Indonesia, India, Tanzania and Brazil (Yahathugoda *et al.*, 2015; Chatterjee *et al.*, 2017; Derua *et al.*, 2017; Oliveira *et al.*, 2017; Vadivalagan *et al.*, 2017). In Malaysia, *Cx. quinquefasciatus* is considered as a nuisance mosquito species (Nazni *et al.*, 2005). Brancroftian filariasis is not the main concern of public health in Malaysia (Vythilingam *et al.*, 2005). However, *Cx. quinquefasciatus* could possibly involve in the local transmission of brancroftian filariasis in the future due to increasing numbers of migrant workers from filariasis endemic regions. *Armigeres subalbatus* are vectors of Japanese encephalitis, filariasis and dirofilariasis to humans (Cheong *et al.*, 1981; Mayhew *et al.*, 2007; Lord *et al.*, 2016).

Diverse species of mosquitoes causes the variability of mosquito breeding habitats in the environment. *Aedes aegypti* prefers to breed in domestic water storage containers inside premises, whereas *Ae. albopictus* immatures are usually found in both natural and artificial containers outside premises (Fulmali *et al.*, 2008; Gautam *et al.*, 2012). Female mosquitoes of both *Culex* *quinquefasciatus* and *Armigeres subalbatus* lay eggs preferentially in stagnant foul water such as in the drainage system as well as in natural and man-made receptacles (Rajavel, 1992; Okiwelu & Noutcha, 2012).

The success of mosquito control strategies depends on the application of proper control methods that should be in line with the mosquito species present within the targeted areas. Entomological surveillance using ovitraps is the most common technique used in determining and monitoring container-breeding mosquito populations mainly *Aedes* (Drago *et al*., 2013; Mackay *et al*., 2013). The ovitrap is often preferred which is attributed to its easiness, handy and inexpensive when used in bulk in the field. The most important feature of the ovitrap is its sensitivity in detecting the existence of *Aedes* population even in low population density.

Previous ovitrap surveillance by local and international researchers had frequently focused on the discovery of *Aedes* populations in human dwellings situated in urban, suburban, rural or remote areas. Very few surveillance studies using ovitraps had been conducted to investigate the container-inhabiting mosquito species especially *Aedes* present in human habitations within agricultural areas. Therefore, the objective of this study was to determine the occurrence and prevalence of single and co-breeding of mosquito larvae especially *Aedes* within human habitations in fogging-free and dengue risk areas in West Malaysia which includes human residences in both residential and agricultural areas.

MATERIALS AND METHODS

Study localities

Mosquito surveillance using ovitraps was conducted in fifteen study sites within West Malaysia. Study sites chosen included human settlements in agricultural areas of oil palm plantations, rubber estates, and paddy growing areas and human settlements within non-agricultural areas involving fogging-free and dengue risk housing areas. Oil palm plantations, rubber estates and paddy growing areas were selected for this study as these crops were the primary industrial crops cultivated in Malaysia (Department of Agriculture Peninsular Malaysia, 2015). Each type of agricultural, fogging-free and dengue risk areas was represented by three study sites, respectively. Agricultural areas selected were ensured to have no reported cases of mosquito-borne diseases such as dengue and chikungunya. However, regular use of pesticides for pest control was noted in these agricultural areas. In contrast, the selection of fogging-free and dengue risk residential areas as study areas was made based on the records of reported dengue cases and history of chemical use in vector control activities in these areas which were obtained from the Ministry of Health Malaysia. The geographical and ecological description of all study sites are provided in Table 1.

Preparation of 10% hay infusion water

Hay infusion water at a dilution of 10% was formulated according to Reiter *et al*. (1991) with some modifications. 41.67 g of dry grass hay was immersed in 5 L of chlorinated-free water for seven days in a 10 L transparent plastic bottle that was entirely protected from light exposure using aluminium foil. After the incubation period, all immersed grass hay were filtered and removed. The prepared 10% hay infusion water was instantly poured into ovitraps.

Ovitrapping of mosquitoes

Ovitrapping of mosquito vectors was performed in each locality using standardized ovitraps as outlined by Lee (1992). Each ovitrap is consisted of a 300 ml black plastic container with an opening and base of 6.8 cm in diameter and 9.1 cm tall. A proper label is glued on the exterior body of ovitrap. A hardboard (10.0 cm x 2.5 cm x 0.3 cm) served as an oviposition paddle for female adult mosquitoes was placed diagonally into each ovitrap with the rough surface upwards. Each ovitrap was added with 10% hay infusion water to a level of 5.5 cm at 24 hours before its introduction in the field.

Table 1. Geographical and ecological description of study sites

State	District	Study areas	Geographical description	Ecological description
		Agricultural area: Oil palm plantations		
Johor	Kota Tinggi	University of Malaya Oil Palm Research Plantation, Jementah **(Kota Tinggi OP)**	• Coordinate: 02º01.727'N, 103º51.924'E • Elevation: 28 m	• An area managed by UM Plantations Sdn Bhd and Boustead Estates Agency Sdn Bhd which consists of a research complex, an administration office and single storey staff quarters within oil palm plantations. • Trees, shrubs, ornamental plants and heavy vegetations could be observed around human dwellings as well as the administration building and the research complex. • Well-built and well-managed water supply system, drainage system and waste management.
Selangor	Klang	Jalan Paip Kiri, Meru **(Klang OP)**	• Coordinate: 03º09.201'N, 101º27.535'E • Elevation: 5 m	• A small residential area consisting of single storey terraced houses located next to an oil palm plantation. • Trees, shrubs, decorative plants and dense vegetations could be observed within the area. • Proper water supply system, drainage system and waste management.
Pahang	Temerloh	Taman Paya Pulai **(Temerloh OP)**	• Coordinate: 03º27.642'N, 102º28.098'E • Elevation: 42 m	• A small and matured residential area comprising single storey terraced houses and located next to an oil palm plantation. • Shrubs, ornamental plants and dense vegetations could be seen around the area. • Appropriate water supply system, drainage system and waste management.
		Agricultural area: Paddy growing areas		
Selangor	Kuala Selangor	Parit 3, Ban 3, Tanjung Karang **(Kuala Selangor PD)**	• Coordinate: 03º29.770'N, 101º09.288'E • Elevation: -25 m	• A rural area with wooden- and brick-made houses scattered along small roads in between rice cultivation fields. • Trees, ornamental plants, vegetations, cash crops and heaps of coconut shells could be seen within compounds of many houses. • Artificial containers such as plastic containers, plastic pails and livestock water tanks could be found inside and outside some houses. • Piped water supply is available but the system to each house is self-built by the home owner. • Improper drainage system and waste management.
Kedah	Kulim	Kg. Terat Batu, Mukim Sidam Kanan **(Kulim PD)**	• Coordinate: 05º32.741'N, 100º32.350'E • Elevation: 9 m	• A rural area with wooden- and brick-made houses scattered within rice cultivation fields. • Trees, ornamental plants, moderate vegetations, shrubs and cash crops could be seen within the area. • Artificial containers such as plastic containers, plastic water tanks and livestock water tanks could be found inside and outside some houses. • Piped water supply is available but the system to each house is self-built by the home owner. • Inappropriate drainage system and waste management.

Negeri Sembilan	Kuala Pilah	Kg. Padang Lebar Terachi, Tanjong Ipoh **(Kuala Pilah PD)**	• Coordinate: 02º44.520'N, 102º07.787'E • Elevation: 81 m	• A rural area with brick- and wooden-made traditional style Malay village houses built on stilts which scattered within small rice cultivation fields. • Most of rice cultivation fields are located at the valley floors, near to the foot of hills. • Trees, decorative plants, moderate vegetations, shrubs and cash crops could be seen around the area. • Heaps of coconut shells and artificial receptacles such as plastic containers, and livestock water tanks could be found inside and outside some houses. • Piped water supply is available but the system to each house is self-built by the home owner. • Inappropriate drainage system and waste management.
		Agricultural area: Rubber estates		
Selangor	Sungai Buloh	Sungai Pelong **(Sungai Buloh RB)**	• Coordinate: 03º12.549'N, 101º32.436'E • Elevation: 39 m	• A village area with wooden- and brick- made houses scattered along small roads surrounding a rubber estate. • Large trees, decorative plants, high vegetations, shrubs and bushes could be seen within the area. • Artificial habitats such as plastic containers and paint buckets could be found within the compound of some houses. • Piped water supply is offered but the system to each house is self-built by the home owner. • Appropriate drainage system and waste management.
Pahang	Temerloh	Taman Jaya 8 **(Temerloh RB)**	• Coordinate: 03º27.423'N, 102º27.638'E • Elevation: 43 m	• A residential area consisting of single storey semi-detached houses located next to rubber estates. • Trees, shrubs, decorative plants and moderate vegetations could be seen within the area. • Proper water supply system, drainage system and waste management.
Johor	Kota Tinggi	Malaysian Rubber Board, Desaru **(Kota Tinggi RB)**	• Coordinate: 01º33.844'N, 104º14.267'E • Elevation: 23 m	• An area managed by Malaysian Rubber Board which comprises of an administration office, working sheds and double storey semi-detached staff quarters situated next to rubber estates. • Trees, shrubs, decorative plants and moderate vegetations could be seen around human dwellings as well as the administration building and working sheds. • Appropriate water supply system, drainage system and waste management.
		Non-agricultural area: Fogging-free residential areas		
Selangor	Shah Alam	Alam Nusantara, Setia Alam **(Shah Alam FF)**	• Coordinate: 03º06.692'N, 101º28.134'E • Elevation: 34 m	• A new residential area comprising of double storey terraced houses and recreation parks. • The environment is generally clean and well-managed. • Young trees and shrubs were planted around recreation parks. • Ornamental plants could be seen placed at the car garage of many houses. • Proper water supply system, drainage system and waste management.

Kedah	Padang Serai	Taman Serai Wangi, Mukim Kulim **(Padang Serai FF)**	• Coordinate: 05º31.301'N, 100º32.673'E • Elevation: 3 m	• A matured residential area consisting of single storey terraced houses, shophouses, and other community facilities. • Big trees, shrubs, ornamental plants and dense vegetations could be observed within the area. • Appropriate water supply system, drainage system and waste management.
Pahang	Temerloh	Taman Seberang Temerloh **(Temerloh FF)**	• Coordinate: 03º26.985'N, 102º26.743'E • Elevation: 19 m	• A new residential area containing of single storey semi-detached houses, recreation parks and other public facilities. • The environment is generally clean and well-managed. • Young trees and shrubs could be seen around the area. • Decorative plants were potted at the car garage and side garden of many houses. • Proper water supply system, drainage system and waste management.
		Non-agricultural area: Dengue prone residential areas		
Johor	Kota Tinggi	Felda Air Tawar 2 **(Kota Tinggi DEN)**	• Coordinate: 01º40.552'N, 104º01.340'E • Elevation: 5 m	• A planned area of the Federal Land Development Authority (Felda) staff quarters comprising of brick- or wooden-made bungalow houses and single storey terrace houses with reported dengue cases each year. • Trees, shrubs, bushes, ornamental plants and high vegetations could be observed within the area. • Piped water supply is offered but the system to each house is self-built by the home owner. • Proper drainage system and waste management.
Selangor	Shah Alam	Kg. Padang Jawa, Seksyen 17 **(Shah Alam DEN)**	• Coordinate: 03º03.000'N, 101º29.200'E • Elevation: 1 m	• An unplanned settlement area consisting of terraced houses, semi-detached houses, bungalows and wooden-made houses scattered along small roads with yearly reported dengue occurrences. • Matured trees, shrubs, bushes and high vegetations could be seen within the area. • Decorative plants and cash crops could be noticed around many houses. • Piped water supply is available but the system to each house is self-built by the home owner or the house developer. • Inappropriate drainage system and waste management.
Federal Territory of Kuala Lumpur	Cheras	Kg. Cheras Baru **(Cheras DEN)**	• Coordinate: 03º06.630'N, 101º45.101'E • Elevation: 89 m	• An established residential area comprising terraced houses, bungalows and wooden-made houses with reported dengue incidents every year. • Matured trees, shrubs, bushes and dense vegetations could be observed within the area. • Ornamental plants could be seen around many houses. • Piped water supply is available but the system varies between different roads. • Proper drainage system and waste management.

By adhering to the guidelines of Ministry of Health Malaysia (1997), 50 ovitraps were deployed in each locality randomly indoors and outdoors which were either partly or completely sheltered to avoid dryness caused by direct sunlight or water spillage caused by heavy rain. In this study, "indoor" refers to the interior segments of the building that are under its roof, while "outdoor" refers to the outside of the building but confined to the immediate surroundings of the building. All ovitraps were put to the closeness to human habitations and other possible natural and artificial breeding grounds of mosquito vectors with minimum physical and environmental distraction. Ovitraps were collected after five days of deployment and conveyed back to the laboratory.

Larval identification

The contents of ovitraps were transferred into respective labeled and covered plastic containers, together with oviposition paddles. Every container was supplied with liver powder (Difco^{TM} Liver; Becton, Dickinson and Company; France) and small chunks of half-cooked cow liver as food for larvae. All hatched larvae were bred up to fourth instar for species identification using standard taxonomic keys by Division of Medical Entomology, Institute for Medical Research (IMR) Malaysia (2000a, 2000b) and Jeffery *et al.* (2012). Number of larvae observed in every positive ovitrap was noted separately.

Data analysis

Data obtained from mosquito ovitrapping conducted were analyzed as follow:

(a) Percentage of positive ovitrap with single breeding: number of positive ovitraps with breeding of individual species against the total of positive ovitraps.

(b) Percentage of positive ovitrap with co-breeding: number of positive ovitraps with co-breeding against the total of positive ovitraps.

(c) Ratio of co-breeding per locality: quotient of the mean number of larvae per recovered ovitrap of the more dominant species against the mean number of larvae per recovered ovitrap of the less dominant species.

One-way ANOVA analysis was carried out using the statistical software programme (IBM SPSS Statistics version 23.0). All levels of statistical significance were ascertained at P=0.05.

RESULTS AND DISCUSSION

The distribution of different species of mosquito larvae in positive ovitraps placed in all study localities is illustrated in Table 2. Sole breeding of *Ae. albopictus* larvae was observed in all study localities with minimum positive ovitraps of 64.3%. In contrast, only two ovitraps (5.6%) deployed at Klang oil palm (Klang OP) showed single breeding of *Cx. quinquefasciatus* larvae while no single breeding of *Ae. aegypti* larvae was detected in any study localities.

For co-breeding, two different species of mosquito larvae were found in certain ovitraps deployed in several study localities. A total of 34 ovitraps collected from five study localities were positive with co-breeding of *Ae. aegypti* with *Ae. albopictus* larvae. *Ae. albopictus* larvae also bred within 32 ovitraps occupied by *Cx. quinquefasciatus* larvae that were deployed in different types of areas. Shared breeding of *Aedes albopictus* larvae and *Ar. subalbatus* larvae was demonstrated in ovitraps located in three paddy growing areas (3 ovitraps), Kota Tinggi rubber estate (Kota Tinggi RB) (6 ovitraps) and Kota Tinggi dengue prone residential area (Kota Tinggi DEN) (1 ovitrap). Moreover, two ovitraps, each of them placed at Kuala Pilah paddy growing area (Kuala Pilah PD) and Kota Tinggi RB were positive with co-breeding of *Cx. quinquefasciatus* with *Ar. subalbatus* and *Ae. albopictus* with *Uranotaenia* sp., respectively.

Overall, co-breeding of two species of mosquito larvae were detected in eleven study localities. Kuala Pilah PD showed the highest number of ovitraps with co-breeding of mosquito larvae (35.7%), followed by Cheras DEN (33.3%). The lowest co-breeding of two different species of mosquito larvae was recorded from Temerloh RB and Temerloh fogging-free residential area (Temerloh FF) with 1 positive ovitrap each.

Table 2. Distribution of sole and co-breeding of mosquito larvae in positive ovitraps recovered from study localities

Status of area	Categories of area	Types of area	Study localities	No. of placed ovitrap	No. of recovered ovitrap	No. of positive ovitrap	No. of positive ovitrap with single species (Percentage, %)		No. of positive ovitrap with mixed infestation of 2 species (Percentage, %)					Total no. of positive ovitrap with mixed infestation of 2 species (Percentage, %)
							Ae. albopictus	*Cx. quinquefasciatus*	*Ae. aegyti* + *Ae. albopictus*	*Ae. albopictus* + *Cx. quinquefasciatus*	*Ae. albopictus* + *Ar. subalbatus*	*Cx. quinquefasciatus* + *Ar. subalbatus*	*Ae. albopictus* + *Uranotaenia* sp.	
Fogging -free area	Agricultural area	Oil palm plantation	Kota Tinggi OP	50	50	40	40(100.0)							
			Klang OP	50	50	35	31(88.6)	2(5.6)	1(2.9)	1(2.9)				2(5.6)
			Temerloh OP	50	50	33	33(100.0)							
		Paddy growing area	Kuala Selangor PD	50	50	38	31(81.6)			6(15.8)	1(2.6)			7(18.4)
			Kulim PD	50	50	33	30(90.9)			2(6.1)	1(3.0)			3(9.1)
			Kuala Pilah PD	50	50	42	27(64.3)			13(30.9)	1(2.4)	1(2.4)		15(35.7)
		Rubber estate	Sungai Buloh RB	50	50	43	36(83.7)		6(14.0)	1(2.3)				7(16.3)
			Temerloh RB	50	50	42	41(97.6)		1(2.4)					1(2.4)
			Kota Tinggi RB	50	50	40	28(70.0)			5(12.5)	6(15.0)		1(2.5)	12(30.0)
	Non-agricultural area	Fogging free residential area	Shah Alam FF	50	50	34	34(100.0)							
			Padang Serai FF	50	50	32	32(100.0)							
			Temerloh FF	50	50	32	31(96.9)			1(3.1)				1(3.1)
Dengue prone area	Non-agricultural area	Dengue prone residential area	Kota Tinggi DEN	50	50	37	33(89.2)			3(8.1)	1(2.7)			4(10.8)
			Shah Alam DEN	50	50	41	31(75.6)		10(24.4)					10(24.4)
			Cheras DEN	50	50	48	32(66.7)		16(33.3)					16(33.3)

Table 3 describes the prevalence of different species of mosquito larvae that shared same ovitraps. All ovitraps that were positive with co-breeding of *Ae. aegypti* with *Ae. albopictus* larvae were predominated by *Ae. albopictus* at more than 5-folds. For co-breeding of *Ae. albopictus* with *Cx. quinquefasciatus* larvae, *Ae. albopictus* were also found to be predominating over *Cx. quinquefasciatus* populations except in ovitraps deployed in Klang OP and Kuala Pilah PD. Moreover, the predominance of *Ae. albopictus* larvae over *Ar. subalbatus* larvae within the same ovitraps was shown in three paddy growing areas. Nevertheless, a contradictory scenario was observed within positive ovitraps with co-breeding of both species deployed at Kota Tinggi RB and Kota Tinggi DEN. Meanwhile, in the ovitrap shared by *Cx. quinquefasciatus* larvae and *Ar. subalbatus* larvae, *Cx. quinquefasciatus* population predominated the ovitrap by 4.8 times, whereas *Ae. albopictus* larvae continued to predominate over the colony of *Uranotaenia* sp. within the shared ovitrap deployed at Kota Tinggi RB by 5.6 times.

Out of 570 positive ovitraps collected from all study localities, 490 of them were occupied solely by *Ae. albopictus*. These results authenticated *Ae. albopictus* as the most prevalent container-inhabiting species in all study localities. The ability of prompt adaptation to diverse natural and man-made breeding grounds by *Ae. albopictus* ensures its endurance in the ecosystem. Meanwhile, *Cx. quinquefasciatus* bred singly only in two ovitraps deployed in Klang OP which showed its partiality to other types of breeding receptacles.

With regard to co-breeding of two or more different species of mosquito larvae within the same ovitrap as noted in this study, this scenario signifies the capability of mosquito larvae to live together within the same ecological settings which supply adequate biotic and abiotic elements for each species of larvae. Previous researchers had also proposed some aspects like geographical and sequential differences, fast and extensive social growth as well as divergence in fecundity and life cycle interval of each species as main causes of co-existence of various mosquito larvae species (Chan *et al.*, 1971; Leisnham & Juliano, 2009).

Co-breeding of various species of mosquito larvae encountered during surveillance performed had been highlighted by many preceding studies throughout the world. In 1971, Chan *et al.* had reported on co-breeding of *Ae. aegypti* with *Ae. albopictus* that occurred at 7.1% and 1.6% of breeding habitats in urban and rural areas in Singapore city, respectively. Co-breeding of *Ae. aegypti*, *Ae. albopictus* and *Cx. quinquefasciatus* in containers was also exhibited in Kolkata, India (Mohan *et al.*, 2014) while Boonklong & Bhumiratana (2016) had described on co-breeding of *Ae. aegypti* with *Ae. albopictus* in various breeding containers found in both urban and rural areas in Narathiwat, South Thailand.

Co-existence of certain species of medically important mosquito larvae had been revealed by local researchers as well. According to Yap & Thiruvengadam (1979), 55.4% of productive ovitraps utilized in Georgetown, Penang Island, Malaysia were occupied jointly by *Ae. aegypti* and *Ae. albopictus* while co-occurrence of both species in urban housing areas (9%) and vacant lands (4.5%) was noticed in Sibu town, Sarawak, Malaysia (Seng & Jute, 1994). Lee (1992) reported that mixed infestation of *Ae. aegypti* and *Ae. albopictus* was found in 3.11% – 8.21% and 5.11% – 9.76% of positive indoor and outdoor ovitraps, respectively in Selangor, Malaysia. More than ten years later, Chen *et al.* (2005) had encountered co-occurrence of *Ae. aegypti* with *Ae. albopictus* populations within same ovitraps placed in two residential areas (4.94% – 6.32%) and a slum area (20.00%) in Selangor, Malaysia. On the other hand, co-existence of *Ae. aegypti* with *Cx. quinquefasciatus* was detected during fourteen months of vector surveillance by ovitrapping conducted in Taman Permai Indah (15.4%) and Kg Pasir Gebu (6.3%) in Penang, Malaysia (Rozilawati *et al.*, 2007). Two years later, co-occurrence of *Ae. albopictus* with *Cx. quinquefasciatus* had been discovered during container survey conducted in a university campus in Kuala Lumpur, Malaysia (Chen *et al.*, 2009).

Table 3. Domination of different species of mosquito larvae in ovitraps positive with co-breeding

Status of area	Categories of area	Types of area	Study localities	Ratio of mixed infestation				
				AE:AL	AL:CQ	AL:AR	CQ:AR	AL:UR
Fogging-free area	Agricultural area	Oil palm plantation	Klang OP	1.0 : 15.6	1.0 : 2.0			
		Paddy growing area	Kuala Selangor PD		2.0 : 1.0	13.0 : 1.0		
			Kulim PD		6.5 : 1.0	1.6 : 1.0		
			Kuala Pilah PD		1.0 : 4.1	21.5 : 1.0	4.8 : 1.0	
		Rubber estate	Sungai Buloh RB	1.0 : 16.3	3.3 : 1.0			
			Temerloh RB	1.0 : 10.8				
			Kota Tinggi RB		7.0 : 1.0	2.1 : 7.0		5.6 : 1.0
	Non-agricultural area	Fogging free residential area	Temerloh FF		2.6 : 1.0			
Dengue prone area	Non-agricultural area	Dengue prone residential area	Kota Tinggi DEN		33.2 : 1.0	1.0 : 8.8		
			Shah Alam DEN	1.0 : 5.4				
			Cheras DEN	1.0 : 8.3				

AE: *Ae. aegypti*
AL: *Ae. albopictus*
CQ: *Cx. quinquefasciatus*
AR: *Ar. subalbatus*
UR: *Uranotaenia* sp.

Similar scenario persisted with co-infestation of *Ae. aegypti* with *Ae. albopictus* in ovitraps being observed in four study areas located in northern and southern regions of Peninsular Malaysia which ranged from 11.36% to 29.03% (Wan-Norafikah *et al.*, 2011). A nationwide larval survey performed by Low *et al.* (2012) had uncovered co-occurrence of *Cx. quinquefasciatus* with *Ar. subalbatus* (1.28% – 3.77%). A year later, Lau *et al.* (2013) performed ovitrap surveillance in high-rise apartments situated in four different residential sites within Kuala Lumpur and Selangor. They successfully spotted co-breeding of *Ae. aegypti* with *Ae. albopictus* in three study sites. However, their findings were in contrary with this study whereby the number *Ae. aegypti* larvae in mixed breeding ovitraps were 1.50–3.44 times higher than *Ae. albopictus* larvae. A larval survey had also been conducted latterly in one similar locality as in this study; Kuala Selangor PD. During the survey, numerous natural and man-made receptacles were found positive with *Ae. albopictus* and *Cx. quinquefasciatus* larvae as well as co-existence of both species (Wan-Norafikah *et al.*, 2017).

Armigeres subalbatus larvae are closely related to natural and man-made receptacles filled with foul water (Rajavel, 1992). Therefore, the foul-smelling of hay infusion water in ovitraps used in this study could had attracted *Ar. subalbatus* to lay eggs in these ovitraps. The use of oviposition stimulants like hay or leaves infusion water had risen the number of eggs in ovitraps (Santana *et al.*, 2006; Santos *et al.*, 2010). The use of different types of detritus could entices assorted species of mosquitoes (Juliano, 2009).

On the other hand, larvae of *Uranotaenia* sp. are generally found in semi-permanent or permanent ground pools (Hinman, 1935) as well as in crab-holes (Peyton, 1970). However, *Uranotaenia* sp. had also been reported to live in man-made receptacles found within cemeteries (Vezzani, 2007). Thus, the detection of *Uranotaenia* sp. larvae in one ovitrap deployed in Kota Tinggi RB confirmed its capability to live in man-made habitats even with the presence of other species of mosquito larvae.

In summary, the co-breeding involving different species of mosquito larvae is persisting throughout the world regardless of the diversity in ecological and meteorological settings. In fact, to the extent of our knowledge, the present study has contributed to a new documented observation on the local co-infestation of *Ae. albopictus* with *Ar. subalbatus* as well as *Ae. albopictus* with *Uranotaenia* sp. The co-breeding scenario shows the ability of more than one species of mosquito larvae to live together within the same environmental settings with no compulsion among them. Consequently, it is crucial for the health department and local authorities to consider the most appropriate vector control approaches to be applied in the field that encompasses all species of mosquito larvae involved in the co-occurrence within same breeding grounds in order to acquire effective control actions.

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