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Quantifying the Distribution and Abundance of Aedes Mosquitoes in Dengue Risk Areas in Shah Alam, Selangor

Anis Hasnan^{a*}, Nazri Che Dom^a, Hazira Rosly^a, Chua Say Tiong^b

^aFaculty of Health Sciences, Universiti Teknologi MARA, 42300 Puncak Alam, Selangor, Malaysia

^bFaculty of Health Sciences, Universiti Teknologi MARA, 13200 Kepala Batas, Pulau Pinang, Malaysia

Abstract

The spread of the dengue disease in Malaysia is related to the abundance of the Aedes population. The aim of the study is to quantify the distribution and abundance of Aedes mosquitoes in both dengue risk area namely controlled and uncontrolled outbreak area. The ovitrap method was applied to quantify the infestation level by calculating the positive ovitrap index (POI) and mean eggs per trap (MET). Finding showed that both POI and MET at uncontrolled outbreak area are higher than controlled area. In conclusion, the ovitrap approach is most sensitive and cost-effective method for detecting the presence of Aedes species.

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Keywords: Positive ovitrap index, mean eggs per trap, dengue

1. Introduction

Dengue is a mosquitoes-borne disease which becomes one of the most prevalent arboviral diseases among the human population. Notably, this disease is now growing rapidly in Malaysia and directly affected the quality of life of the community. Dengue fever (DF) is endemic in Malaysia and has seen a recent resurgence despite and effective vector control program based on a three-pronged approached that incorporates source reduction, public health

* Corresponding author. Tel.: +60332584447; fax: +60332544457.

E-mail address: nazricd@salam.uitm.edu.my

education and law enforcement (Md Shahin et al., 2013). The annual report by Ministry of Health (MOH) of Malaysia documented that over 120,836 positive cases were reported in Malaysia in the year 2015, and 11.2% increases over that reported in the year 2014. In congested to that, Selangor was recorded the highest numbers of DF cases as compared to the other states in Malaysia.

This disease has been declared as the national health threats to the public in Malaysia. DF irrespectively attacks all age group with the most vulnerable among the school going children and young adults. This can be attributed to the young generation lifestyles where they are physically active and spending more outdoor than indoors. Besides that, the study conducted in China revealed that the continuous process of urbanization has contributed to the incremental of the dengue incidence (Ho et al., 2005). The recent studies have proposed that the dengue has spread from urban city centers to the more rural populations, including to the forest fringe areas where the majority of the aboriginal population resides (Oyewole et al., 2009; Nazri et al., 2012). The Ministry of Health of Malaysia summarized that the increasing of dengue were due to the neglecting of having the good quality of living environment including unhygienic conditions, irresponsible among the denizens by littering the rubbish everywhere, and non-systematic waste management where it is favorable for the *Aedes* mosquitoes' infestation.

Aedes mosquitoes are responsible in transmitting DF, where these vectors are increasing their geographical distribution and thus allowing the entry of viruses into new populations of susceptible human hosts (Hsueh et al., 2012). The population dynamics of the adults *Aedes* mosquitoes is highly significant in the epidemiology of the dengue control, where the abundance of the dengue vectors are influenced by the female behavior oviposition and the survivals of the progeny. Thus, the adult densities of *Aedes* mosquitoes have multiplied due to the factors mentioned above. The abundance of these dengue vectors is linked with the biotic and abiotic factors. According to Sirpia et al. (2013), the spatial distribution and abundance of the dengue vectors are related to the effects of anthropogenic changes in the environment. Otherwise, the distribution of dengue vectors is more associated with the presence of the vegetation in urban and rural areas, and its abundance relies on spaces modified by human activity. The environmental variables, such as rainfall, temperature, and relative humidity also play a vital role in influencing population density of the *Aedes* mosquitoes. This is proved by Focks et al. (1993) which claimed that the meteorological factors may affect the mosquito metabolism oviposition activity, thus influence the number of eggs laid by the female. Previously, Azil et al. (2010) documented that bioecological aspects can be measured in positive ovitraps and egg density.

Ovitraps surveillance is very beneficial for planning and managing dengue vector. This approach also is a useful tool in providing spatial and temporal data for monitoring the impact of control measures (Ligia et al., 2013).

Besides that, the utilization of ovitraps is recommended for differentiating the infestation levels between the areas, especially in high risk areas. Most of the research focuses on the distribution and abundance of the *Aedes* population in dengue hotspot areas as well as randomly chosen the areas as the study area. But few studies attend to characterizing and investigate on the abundance of *Aedes* mosquitoes between uncontrolled dengue outbreak area and controlled dengue outbreak area. Therefore, this study aims to determine the distribution and abundance of *Aedes* mosquitoes in the central zone of Shah Alam with respect to different dengue risk areas namely controlled and uncontrolled outbreak areas.

2. Methodology

2.1. Study area

This study was concentrated in Shah Alam (3.0833° N, 101.5333° E) in order to quantify the distribution and abundance of the *Aedes* mosquitoes which influences the transmission of the dengue infection. The area located 25 kilometers from the capital city of Kuala Lumpur which covering approximately 290.3 km² of Selangor state (Md Shahin et al., 2013). The high population in Shah Alam (2000 residents/km²) is the driven factors to the high occurrence of the dengue outbreaks where the number of cases increased yearly. The incremental of cases was due to the improper condition of the surrounding environment. The incremental trend of dengue cases in this area was significantly related to the public health implication in relation to the control and dengue prevention. Therefore, in this study, the surveillance was focused on the central zone of Shah Alam, which covered Seksyen 1 to Seksyen 24.

This region is highly dense and undergoes rapid development as compared to the other zones. Besides that, the region also recorded high DF cases as shown in Figure 1.

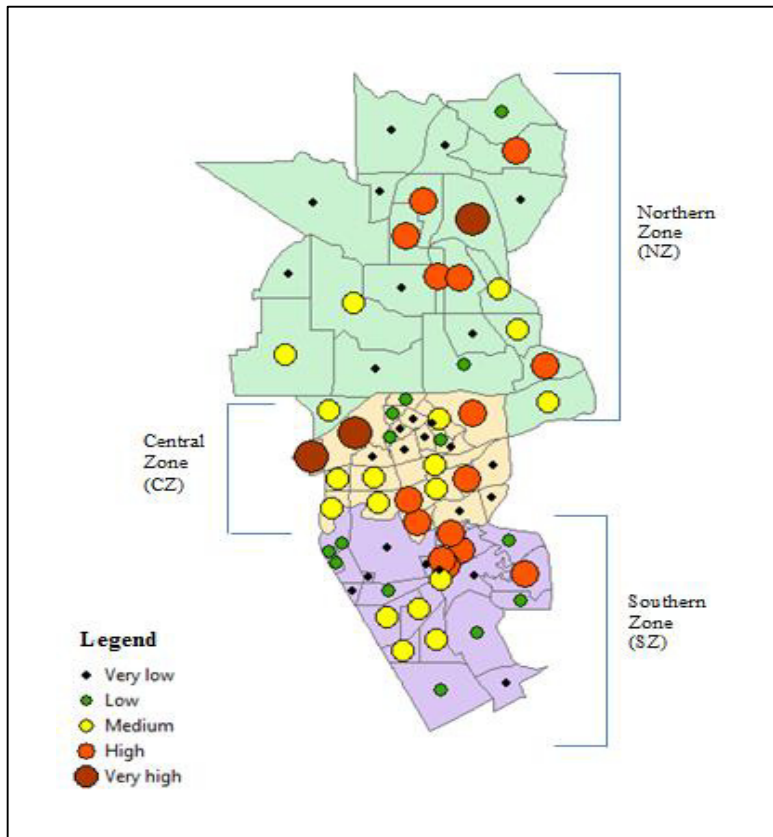


Fig. 1. The spatial distribution of dengue cases in Shah Alam, year 2012 to 2014

2.2. Data collection and identification of dengue risk areas

Operationally this study had defined dengue risk area namely (i) dengue controlled outbreak (DCO) area and (ii) dengue uncontrolled outbreak (DUO) area. Dengue controlled outbreak area is defined as the outbreak which happens when there are no new dengue cases notified after 14 days. Conversely, uncontrolled outbreak area defined outbreak which happens when there is a new notification of dengue cases after 14 days (MOH, 2015).

In this study, the epidemiological data on daily DF cases from the year 2010 to 2014 which include the onset data, place of the notified DF cases, and laboratory test date was obtained from the Vector Control Unit, Petaling District Health Office. Spatial distribution of the dengue cases was constructed on the basis of the two different risk areas as mentioned above, by using the data of DF cases from the year 2010 to 2014. Table 1 shows the localities, based on the different dengue risk area namely controlled outbreak area and uncontrolled outbreak area.

Table 1. Study localities based on the dengue cases reported in Shah Alam, from year 2010 to 2014

Spatial indices	Localities	Abbreviation	Coordinate	DF episode
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			Latitude	Longitude	
Dengue controlled outbreak (DCO) area	Seksyen 1	S1	3.11°	101.554°	11
	Seksyen 4	S4	3.09°	101.535°	17
	Seksyen 6	S6	3.08°	101.519°	20
	Seksyen 8	S8	3.08°	101.511°	18
	Seksyen 9	S9	3.08°	101.533°	15
	Seksyen 15	S15	3.05°	101.517°	22
	Seksyen 20	S20	3.04°	101.557°	19
Dengue uncontrolled outbreak (DUO) area	Seksyen 3	S3	3.08°	101.521°	8
	Seksyen 7	S7	3.08°	101.522°	6
	Seksyen 17	S17	3.09°	101.554°	12
	Seksyen 18	S18	3.05°	101.516°	15
	Seksyen 24	S24	3.04°	101.554°	10

2.3. Ovitrap setting

The ovitraps used in this study consist of a black plastic container filled with a mixture of water and hay infusion (150 ml: 20 ml), and a paddle made of wooden hardboard (8 cm x 2 cm) to collect the eggs. The traps were used and positioned vertically to provide at gravid females a surface for oviposition. In order to standardize the environmental parameters and to avoid the differences in the attractiveness, the ovitraps were placed in the suitable habitats for the presence of the mosquitoes as illustrated in figure 2 below. In the field, the dechlorinated water was used as the water mixture in the ovitraps to provide a favorable habitat for the mosquitoes to lay eggs. In the two different risk areas, the ovitraps were distributed inside 12 localities and were georeferenced in the field using GPS and identified with the code. The traps remained exposed for four consecutive days. Two traps were positioned in each site at a distance of 150 to 200 m from each other. This study was conducted during the transition period (November-December) where it is the time of the early rainy season, by the other word; it is the end of the dry season and the beginning of the rains.

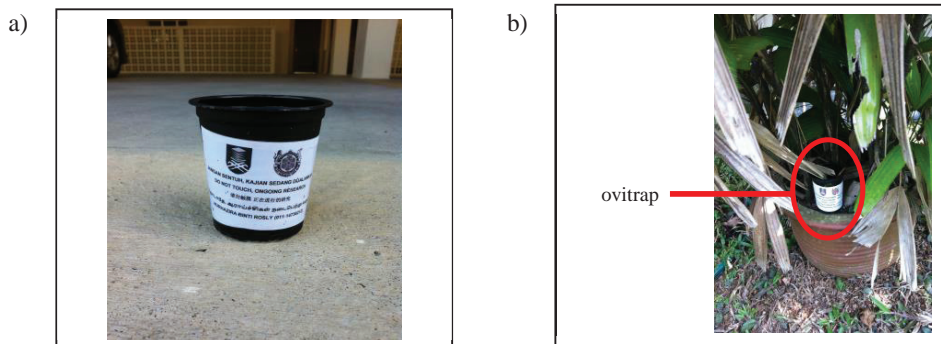


Fig. 2. a) the ovitrap used; b) placement of ovitrap at the favourable breeding site

2.4. Ovitrap collection and identification of eggs

After four consecutive days, the ovitraps were collected back. The paddles were transferred into the airtight plastic (13 cm x 6 cm). Meanwhile, the ovitraps were tightly covered with its container cap. This process is carried out with caution to avoid the water spills occurred and resulted to the loss of the eggs. The paddles were then dried in advance to facilitate the process of eggs identification. The number of eggs in the water also was taking into account as the eggs might be in the water. The water in the ovitraps were filtered using the filtered paper and were dried for the eggs identification. Both paddles and filtered paper (Figure 3) were examined by stereomicroscope at the Vector Control Research Laboratory of Faculty Health Science in UiTM for the eggs counting. The ovitraps density was decided upon according to Emilia Romana guidelines and without previous monitoring data.



Fig. 3. *Aedes* mosquitoes' eggs on filter paper were examined under stereomicroscope

2.5. Data analysis

To measure the population abundance of *Aedes* mosquitoes' eggs, the entomological indices recommended by the Ministry of Health were calculated such as positive ovitrap index (POI) and mean eggs per trap (MET). The POI value represents the mosquitoes' distribution while MET value indicates the vector's population abundance. The descriptive analysis was performed in order to understand the distribution and abundance of *Aedes* mosquitoes in both dengue risk areas.

$$\text{Positive Ovitrap Index (POI)} = \frac{\text{Number of positive traps}}{\text{Number of inspected traps}} \times 100 \quad (1)$$

$$\text{Mean egg per trap (MET)} = \frac{\text{Number of eggs}}{\text{Number of positive traps}} \quad (2)$$

3. Results

In total, 360 ovitraps were deployed, and 256 were recovered, and 216 (84.4%) ovitraps showed the presence of the *Aedes* sp. Eggs, including 99 (45.8%) positive ovitraps in controlled outbreak area and 117 (54.2%) ovitraps in the uncontrolled outbreak area. Dominantly, positive ovitraps were detected in uncontrolled outbreak area even though the numbers of localities from this risk area is lesser compared to controlled outbreak areas. The immersion of all these positive ovitraps enabled the estimation of the *Aedes* mosquitoes' distribution. This indicates the mosquito's abundance is higher in uncontrolled outbreak area as compared to the controlled area.

The differences of dengue risk areas also influence by the vector's population as shown in figure 4.a. The numbers of eggs collected were greatly higher in uncontrolled outbreak area (2529 eggs (74.1%)) as compared to

controlled outbreak area (884 eggs (25.9%)). This distribution of the number of eggs, according to the different risk area, corresponded to the 45.8% and 54.2% of positive ovitraps for controlled outbreak area and uncontrolled outbreak area, respectively.

Overall, all the localities grouped in uncontrolled outbreak area presented higher positive ovitrap index (POI) with the highest index showed by S17 (87%) and S24 (87%). The highest POI value in controlled outbreak area, shown in S9 (79%). Meanwhile, the highest mean egg per trap (MET) that the female mosquito oviposits on a single trap was recorded in uncontrolled outbreak area represented in S18 (Mean eggs/trap: 32.86). Figure 4.b clearly presented the trend of the POI and MET of both risky areas. Both POI and MET value of uncontrolled outbreak area illustrated clearly by the graph to have a higher value compared to the controlled outbreak area. The most susceptible locality showed by the graph is S18 where both POI and MET value are among the highest from all.

a)

Risk area	Locality	Ovitrap placement	No. of recovered ovitrap	No of positive trap (+)	Ovitrap index (%)	No. of eggs	Mean eggs per (+) trap
Dengue controlled outbreak (DCO) area	S1	30	29	6	21.0	142	23.67
	S4	30	29	16	55.0	152	9.5
	S6	30	30	14	47.0	95	6.78
	S8	30	30	8	27.0	88	11.0
	S9	30	29	23	79.0	209	9.09
	S15	30	30	17	57.0	119	7.0
	S20	30	30	15	50.0	79	5.26
	Total	210	217	99	Total	884	
Dengue uncontrolled outbreak (DUO) area	S3	30	30	21	70.0	399	19.0
	S7	30	29	22	76.0	441	20.04
	S17	30	30	26	87.0	540	20.79
	S18	30	30	22	73.0	723	32.86
	S24	30	30	26	87.0	426	16.38
	Total	150	149	117	Total	2529	

(b)

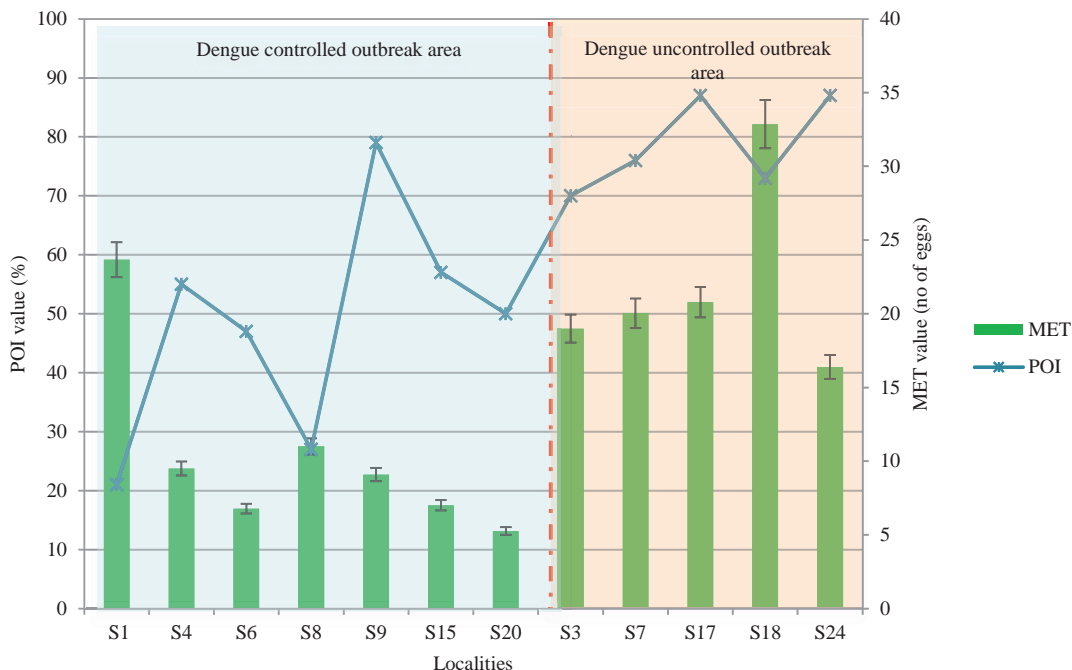


Fig. 4. a) Table of ovitrap placement, numbers of recovered ovitrap, number of positive ovitrap, ovitrap index, number of eggs and mean eggs per trap of trap; b) The graph of POI and MET with respect to the localities of different risk area

4. Discussion

This study utilized the ovitraps to quantifying the abundance of the *Aedes* mosquitoes abundance and its population density in two different dengue risk areas namely controlled outbreak area and uncontrolled outbreak area. These comparisons of this vector population in different risk area are still unidentified as the previous studies are more likely to investigate the random selected or hotspot areas to be included as their study area of concerned. Due to the dynamic growth of dengue cases in Malaysia, it is important to investigate the distribution of the *Aedes* mosquitoes' population performances. From this study, it is clearly shown that the *Aedes* is greater in the uncontrolled outbreak area. The long episode of dengue outbreak in this area may be due to the high population of its vector. This hypothesis could be further investigated as the dengue cases are at alarming in this country.

The abundance of the *Aedes* mosquitoes in the human living environment is suspected due to the neglecting to the surrounding environment and invited to the unhygienic conditions. The irresponsible of the people in managing their surrounding may contribute to the favorable condition of the mosquitoes breeding site (Md Shahrin et al., 2013). Besides that, the high abundance of the dengue vector is directly affected the daily life of the human kind. The nature of *Aedes* that used to live near to human as they can have meals by feeding the blood gives nuisance to the person for example during the dusk and dawn which the time of *Aedes* mosquitoes to be active. These can decrease the quality of human to have a good sleep (Lu et al., 2009). Besides that, the abundance of the *Aedes* can limit the physical and outdoor activities where the transmission of the dengue viruses could be more in chances. A study by Focks et al. (1993), the *Aedes* behavior tends to attract the pheromone of the active person. Besides that, high presence of *Aedes* in human surrounding would seriously increase the transmission of the dengue virus to human and the adaptation of the *Aedes* to the human environment is proven.

Some researchers suggested that the deposition of the eggs by the *Aedes* mosquitoes was significantly related to the climatic factors. Nazri et al. (2013) suggested that the temperature and humidity are the core factors to influence the *Aedes* mosquitoes' performances. The increase of minimum temperature and decreased wind velocity were associated with the increasing rate of dengue cases. In another study, Rueda et al. (2004) documented that the relative humidity affects the survivals of mosquito's eggs and the adults. Newly laid eggs are subject to desiccation and the adults to moisture-related reductions in survivals throughout their lifetimes. Besides that, at all stages of the life cycle, the strongest humidity reaction was an avoidance of high humidity above 95 percent R.H (Nazri et al., 2013). The development cycle of mosquito in the rainy season was the quickest. Apart from the time taken for the developmental cycle, the number of adults emerging at the end of it was lower in the hot season than the cold season (Thu, Aye, & Thein, 1998). The situation of dry weather and the increasing of minimum temperature, the mosquitoes' life cycle from eggs to adult could be increased during that period which is less than normal seven days of its life cycle thus enhance the increasing of dengue vector population (Lu et al., 2009).

Nowadays, the economic burden could increase as the double cost of the vector control measures is considered. The control of dengue vectors has been discussed by several people (Focks et al., 1993; Lu et al., 2009; Oyewole et al., 2009) and the control of DF mostly authoritatively presented in WHO (1975). Many methods are available to be adopted for the control of the dengue vectors, e.g. environmental control, chemical control, biological control, genetic control, human behavioral control and others. Of this method, human behavioral control, environmental control and efficient vector surveillance was considered to be the most effective on a long-term basis since they have the same aim focus which to eliminate and reduces the number of *Aedes* breeding sources (Nazri et al., 2013). Human behavior control dealing with the habits of the human to prevent and control the propagation of mosquitoes in the premises. People can be encouraged to participate in *Aedes* control program through the effective health education and the community participation the project of clean up the compounds and removes mosquito breeding sources. Besides that, in education people for better living the coercion and enforcement could be operationally enhance the preventive and control measures. Piyaratne et al. (2005) claimed that health education could together go along with law enforcement and boost up the efficient tools in dengue fever prevention. Since DF is a man-made disease, human behavioral control is undoubtedly the most important and effective measure in the long-term battle against the dengue vectors.

The arbovirus transmission among human is high, although it is believed to depend on the habits and densities of the species involved. The practical and operational methods using ovitraps may provide such information, which is beneficial for planning and managing the vector. In the situations where population density is low, the ovitraps has proven to be effective in the study of the spread of the vectors and resistance to the insecticides thus can assess the impact of the vector control measure (Fillinger et al., 2008). Ovitrap uses were recommended for differentiating infestation level between areas. The ovitrap data also have been successful to monitor the impact of the various types of control measures involving source reduction and insecticides application, (Focks, 2003). Also, the ovitrap method is capable of detecting the mosquitoes from unexposed breeding sites and surrounding areas.

Environmental control is the method to control the DF outbreak permanently through the improvement of the environmental condition (WHO, 1975). The principle involved either the destruction of immature stages or disruption of the mosquito immature life cycle. These can be effectively achieved through public participation in guided by the health authorities in motivating the public to contribute actively to vector control activities. Vector surveillance is one of the most important aspects of the vector control. It is essential step in the surveillance of the vector-borne disease. The purpose of the surveillance is to determine the presences of vectors, the infestation rate, abundance and distribution and other epidemiological parameter relating to vectorial capacity. It is utmost importance to have continuous basic information on the distribution and density of the vectors. Therefore, routine larval surveys in all built-up areas would pinpoint the high *Aedes* infestation and routine plotting of DF cases on map would pinpoint the high disease endemic areas. Thus, routine, year-round vector and disease surveillance would enable to identify priority areas to be periodically mapped out for control. It must be emphasized that all the above methods, by themselves are not entirely effective. An integration of them is necessary for reducing and eliminating the vector breeding sources thus achieves effective control of DF outbreak. Needless to say, a control strategy must be developed well before an outbreak occurs. Therefore, these integrated methods are needed and should be carried out the whole year round.

5. Conclusion

The depositions of eggs of this vector occur homogeneously in the two dengue risk area. This study claimed that the uncontrolled outbreak areas seem to be higher as compared to controlled outbreak area. This finding provides the health agencies to estimate the infestation rates thus enhance the vector control program to the optimal level of effectiveness thus provide the good quality of life among the population.

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References

- Arunachalam, N., Tana, S., Espino, F., Kittayapong, P., Abeyewickrem, W., Wai, K. T., Tyagi, B. K., Kroeger, A., Sommerfeld, J., & Petzold, M. (2010). Eco-bio-social determinants of dengue vector breeding: a multicounty study in urban and peri-urban Asia. *Bulletin of the World Health Organization*, 88, 173-184.
- Azil, A. H., Haile, D. C., Daniels, E., & Mount, G. A. (2010). The development of predictive tools for pre-emptive dengue vector control: a study of *Aedes aegypti* abundance and meteorological variables in North Queensland, Australia. *Trop Med Int Health* 2010. 1190-1197.
- Fillinger, U., Kannady, K., William, G., Vanek, M. J., Dongus, S., Nyika, D., Geissbühler, Y., Chaki, P. P., & Govella, N. J. (2008). A toolbox for operational mosquito larval control: preliminary results and early lessons from the Urban Malaria Control Programme in Dar es Salaam, Tanzania. *Malaria Journal*, 7, 20-28.
- Focks, D. A., Haile, D. G., Daniels, E., & Mount, G. A. (1993). Dynamic life table model for *Aedes aegypti* (Diptera: Culicidae): simulation results and validation. *Journal of Medical Entomology*, 30, 1018-1028.
- Focks, D. A. (2003). A review of entomological sampling methods and indicators for dengue vectors. Special program for Research and Training in Tropical Disease (TDR) UNICEF/UNDP/WORLD BANK/WHO, Geneva, 1-40.
- Ho, C. Feng, C. Ta-Yang. (2005). Surveillance for dengue fever vectors using ovitraps at Kaoshiung and Tainan in Taiwan. *Formosan Entomol*, 25, 159-174.
- Ligia, L. N. S. G., Ana, P. L., Julio, C. V., & Marylene, B. A., V. G. V. (2013). Study of the distribution and abundance of the eggs of *Aedes aegypti* and *Aedes albopictus* according to the habitat and meteorological variables, municipality of Sao Sebastiao, Sao Paulo State, Brazil. *Parasites & Vectors* (2013), 6, 321.
- Lu, L., Lin, H., Tian, L., Yang, W., Sun, J., & Liu, Q. (2009). Time series analysis of dengue fever and weather in Guangzhou, China. *BMC Public Health*, 9(1), 395.
- Md. Shahin, M., Rawshan, A. B., Raja, D. Z. R. Z. A., & Joy, J. P. (2013). Trends of dengue infections in Malaysia, 2000-2010. *Asian Pacific Journal of Tropical Medicine* (2013), 462-466
- Murrell, E. G., & Steven, A. J. (2008). Detritus type alters the outcome of interspecific competition between *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae). *Journal of Medical Entomology*, 45(3), 375-385.
- Nazri, C. D., Latif, Z. A., Ahmad, A. H., Ismail, R., & Pradhan, B. (2012). Manifestation of GIS tools for spatial distribution analysis of dengue fever epidemic in the city of Subang Jaya, Malaysia. *Environment Asia*, 5, 82-92.
- Nazri, C. D., Hashim, A., Rodziah, A. H., & Abu Yazid, A. (2013). Utilization of geoinformation tools for dengue control management strategy: A case study in Seberang Prai, Penang Malaysia. *International Journal of Remote Sensing Application*, 3, 11-17.
- Nazri, C. D., Rodziah, A. H., & Ismail, R. (2013). Habitat characterization of aedes sp. breeding in urban hotspot area. *Procedia – Social and Behavioral Sciences*, 85, 100-109.
- O'Malley, C. (1995). Seven ways to a successful dipping career. *Wing Beats*, 6, 23-24.
- Oyewole, I.O., Momoh, O. O., Anyasor, G. N. (2009). Physico-chemical characteristics of Anopheles breeding sites: Impact on fecundity and progeny development. *African Journal Environmental Science Technology*, 3, 447-52.
- Paupy, C., Delatte, H., Bagny, L., Corbel, V., & Fontenille, D. (2009). *Aedes albopictus*, an arbovirus vector: from the darkness to the light. *Microbes Infection*, 11, 1177-1185.
- Piyaratne, M. K., Amerasinghe, F. P., Amerasinghe, P. H., & Konradsen, F. (2005). Physico-chemical characteristics of Anopheles culicifacies and Anopheles varuna breeding water in a dry zone stream in Sri Lanka. *Journal Vector Borne Disease*, 42, 61–67.

- Rueda, L. M. (2004). *Pictorial keys for the identification of mosquitoes (Diptera: Culicidae) associated with dengue virus transmission*. Walter Reed Army Inst of Research Washington, Department of Entomology.
- Thu, H. M., Aye, K. M., & Thein, S. (1998). The effect of temperature and humidity on dengue virus propagation in *Aedes aegyptimosquitos*. *Tropical Medicine & International Health*, 6(9), 677-687.