Mosquito larvae prevalence at Suranaree University of Technology, Nakhon Ratchasima,Thailand

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Abstract: Mosquitoes transmit several of diseases to humans. Their abundance and distribution mostly related to the characteristics of larval habitats. This study investigated the prevalence of mosquito larvae in two different areas at Suranaree University of Technology (i.e. dormitory and academic area). Mosquito larvae samples were collected from 30 households in two different areas during August-November 2017. There were five mosquito larvae species (i.e. *Ae. aeqypti*, *Ae. albopictus*, *Culex* sp., *Armigeres* sp. and *Toxorhynchites* spp.) were found in total number of 3,183. *Armigeres* sp. was found in the highest number mostly in cement tanks, follow by *Culex* larvae which were found mostly in waste plastic containers and small earthen jars sp., *Ae. Aegypti* and *Ae. albopictus* larvae were found mostly in flower vases, and *Toxorhynchites* spp. larvae were found mostly in small earthen jars and coconut shells, respectively. All three *Aedes* larvae indices: Container Index (CI) for *Aedes* spp. was >5%, House Index (HI) for *Aedes* spp. was >5% and Breteua Index (BI) for *Aedes* spp. was >20 within both dormitory and academic areas, which indicating that dormitory area are dengue sensitive. We also found that the all three larvae indices in academic area was higher than dormitory area. These results are essential for developing the effectively larval control strategies in SUT.

Keywords: mosquito larvae, breeding sites, *Aedes* spp., *Culex* spp., *Armigeres* spp., *Toxorhynchites* spp.

Introduction: Mosquitoes are extensively distributed worldwide, there have been found throughout the tropics and temperate regions (Rueda, 2008; Nikookar et al., 2017). Mosquitos transmitted virus to infect in animal and cause human diseases such as dengue, malaria, chikungunya, filariasis, and Japanese encephalitis (Gopalakrishnan et al., 2017). The most important pest and vector species belong to the genera *Aedes*, *Anopheles*, *Culex*, *Psorophora*, *Haemagogus* and *Sabethes* (Service, 2000; Saleeza, 2013). *Ae. agypti* is the essential vector of dengue and yellow fever (Gopalakrishnan et al., 2017; Adelman et al., 2002). *Ae. albopictus* is the vector of dengue and chikungunya (Giatropoulos et al., 2012). *Culex* sp. is the vector of lymphatic disease, filariasis, Japanese encephalitis (Rattanarithikul et al., 2005). *Armigeres* is a vector of nematode parasites that cause lymphatic filariasis (Shi et al., 2014). *Aedes aegypti*, *Ae. albopictus* and *Culex quinquefanciatus* were the most predominant species among the container breeding mosquitoes (Rattanarithikul et al., 1994; Chumsri et al., 2015). The breeding sites of mosquito larvae have unique ecological properties and these habitat could be natural such as tree holes and leaf axils (Thangamathi et al., 2014) or artificial such as large earthen jars, cement tanks, bowl, used cans and discarded tyres (Chumsri et al., 2015).

Due to these mosquito are important from the public health perspective as they include disease vectors as well as potential biological control agents (Chumsri et al., 2015). In Thailand, the Department of Disease Control reported there are 9,614 case of Dengue Hemorrhagic Fever (DHF) in northern east Thailand, with three deaths in 2016. Moreover, the risk of dengue disease is highest in 15-24 year olds, follow by 10-14 year olds, the most of that age are students (Department of Disease Control, 2018). However, most of studies have been focusing on mosquito larvae breeding sites, habitat, and abundance in residential, community, urban or rural area (Rueda, 2008; Saleeza, 2013; Chumsri, 2015; Nikookar et al., 2017). Very few reported of mosquito larval prevalence in schools. Therefore, this study examined how key breeding sites of mosquito larvae species vary among two different locations (i.e. dormitory and academic area) in Suranaree University of Technology.

Materials and Methods:

Study site: Mosquito larval survey was conducted in two different workspace (i.e. dormitory area and academic area) at Suranaree University of Technology, Nakhon Ratchasima, Thailand (14.88134˚ N and 102.02066˚ E) during August - November 2017. The Suranaree University of Technology (Thai: มหาวิทยาลัยเทคโนโลยีสุรนารี, (SUT) is in Nakhon Ratchasima Province, Thailand. The university was established on 27 July 1990, becoming fully operational in 1993. It is one of nine National Research Universities of Thailand. The university has a trimester system, consisting of 13 weeks for each trimester. There are about 16,498 students in academic year 2017 and 2,163 academic staff members. There university has an area about 1120 hectares. Mosquito larvae samples were collected from of the dormitory area in total 13 buildings and 17 buildings of academic area (Figure 1).

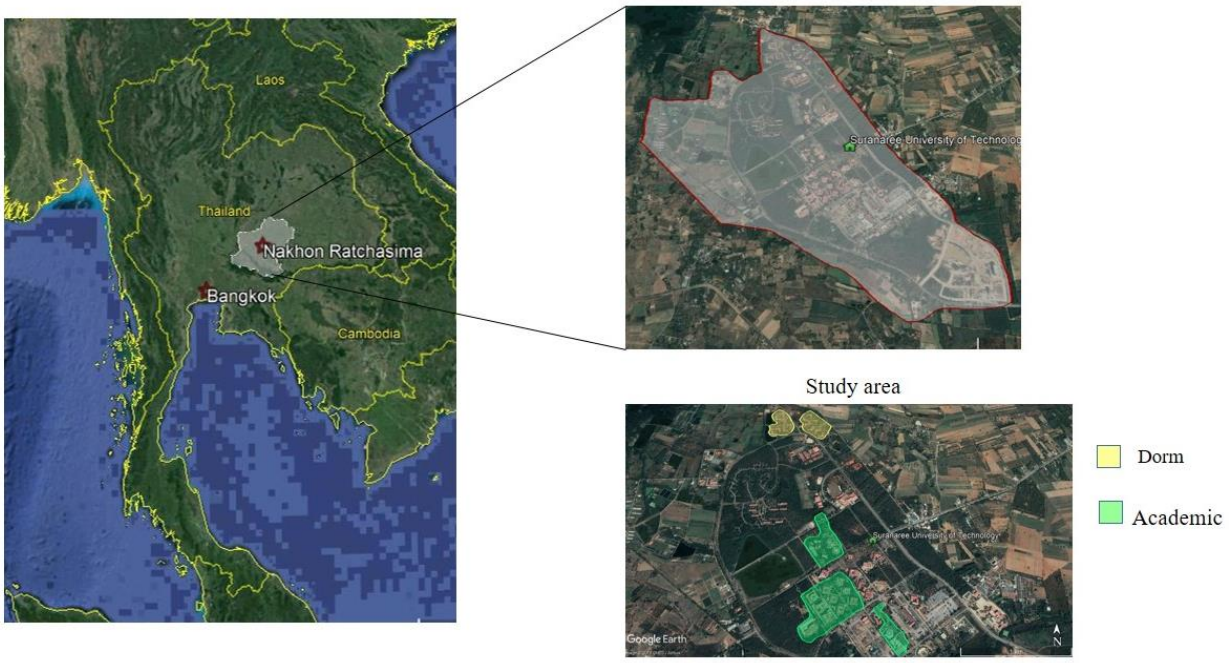


Figure 1 Location of the study site. Suranaree University of Technology, Nakhon Ratchasima, Thailand.

Entomological studies: Larval survey were conducted in the study area by using fishnet of 11.5 diameter size (Wongkoon et al., 2005). Breeding placed were sampled in both indoor and outdoor within 15 m of the households (Raju, 2003). All larvae breeding in small containers were emptied through the fishnet into the bucket. Large containers were sampled by dipping the net in the water, starting a swirling motion and sampling all edges of the container (Strickman et al., 2003; GLOBE protocols, 2017). All live mosquito larvae were collected in plastic bags, brought to laboratory for identification up to the species level by using GLOBE mosquito protocols and Rattanarithikul and Panthusiri keys (Rattanarithikul et al., 1994; GLOBE protocols, 2017). In this study, the first, second instars, and pupae were discarded because immature mosquitoes at these stages could not be identified (Chumsri et al., 2015). Mosquito data were input to the GLOBE website (https://www.globe.gov/globe-data/data-entry) under GLOBE Hydrology protocols.

All three larval indices: House Index (HI), Container Index (CI), and Breteau Index (BI) were worked out as per standard WHO guidelines. HI has been widely used to calculate the presence and distribution of *Aedes* spp. population in a given locality but does not take into consideration the number of positive containers per house. CI provides information on the proportion of water containers that are positive. BI establishes a relationship between positive containers and number of houses. The BI and HI are commonly used for determining of priority (risk) areas for control measures, if HI greater than 5% or HI greater than 20 for any locality is indicated that these area are dengue-sensitive. (WHO, 2003; Chumsri,2015).

Statistical analysis: All data were tested for normality using the Komogorov-Smirnov test. Differences in the numbers of different species of mosquito larvae from different study sites were tested by using crosstab chi-square test, and differences in the number of different species of mosquito larvae from each site was tested by using chi-square test. The number of positive containers in two different locations were compare using independent sampled *t*-test.

Results:

Dormitory's and academic's water containers: For the dormitory's containers, there were plastic tank, flower plastic vases, plant water pot, cement tank, coconut shells, small earthen jars, and artificial pond (Figure 2a). For containers of academic area, there were flower plastic vases, plastic tank, others (e.g. artificial pond, bottle used, cup used, water bucket, natural pond by track of wheel, large earthen jar), cement tank, small earthen jars, flower glass vases, plant water pot, pottery vases, paint bucket, wastes containers and bowls (Figure 2b). There were differenced in the number of positive containers between study areas (t_{51} = 4.050, P<0.001). Differences mosquito larvae species were found in variety of water containers (Figure 3 a-b).

***Armigeres* sp.** larvae were found only in academic area with the highest amount, and found mostly in cement tanks (Figure 3b).

Ae. aegypti larvae were found only in academic area, and found mostly in flowers plastic vases, plastic tanks and flower glass vases (Figure 3b).

Ae. albopictus larvae were found in both dormitory and academic area, and found in flower plastic vases, coconut shells, flower ceramic vases, plastic tanks, plant flower pot, pottery vases and paint bucket (Figure 3a-b).

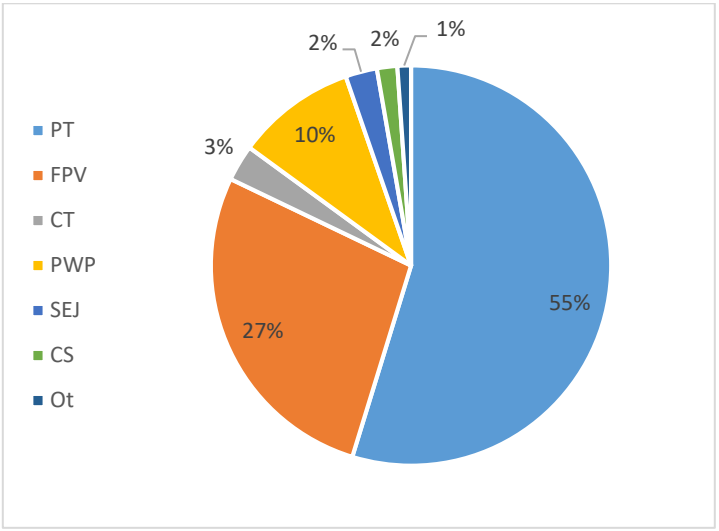
***Culex* sp.** larvae were found in waste containers, small earthen jars, cement tanks, plastic tanks, flower plastic vases and coconut shells (Figure3a-b).

***Toxorhynchites* spp**. larvae were found in small earthen jar and coconut shells (Figure 3a-b).

Mosquito larvae species in different study sites: There was an interaction between study areas and the number of different mosquito larvae from different locations (crosstab chi-square: $X_4^2 = 372.105$, $P<0.001$). *Ae. aegypti*, *Ae. albopictus*, *Culex* sp. and *Armigeres* sp. larvae in academic area were higher number of mosquito larvae than dormitory area (Table 1).

Larval indices: House index (HI) of *Aedes* spp. was >5% in both study areas. Breteau Index (BI) for *Aedes* spp. was >20 in both of study areas, indicating that all of workspace are dengue sensitive (Table 2).

(a)



(b)

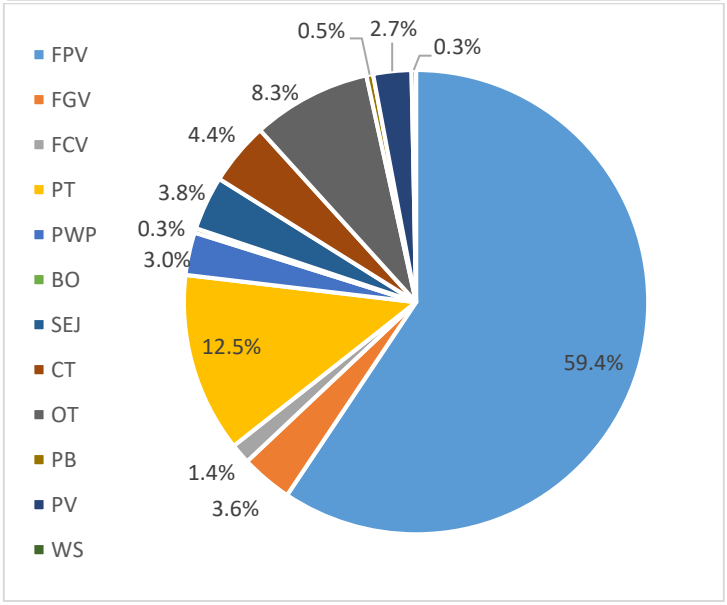
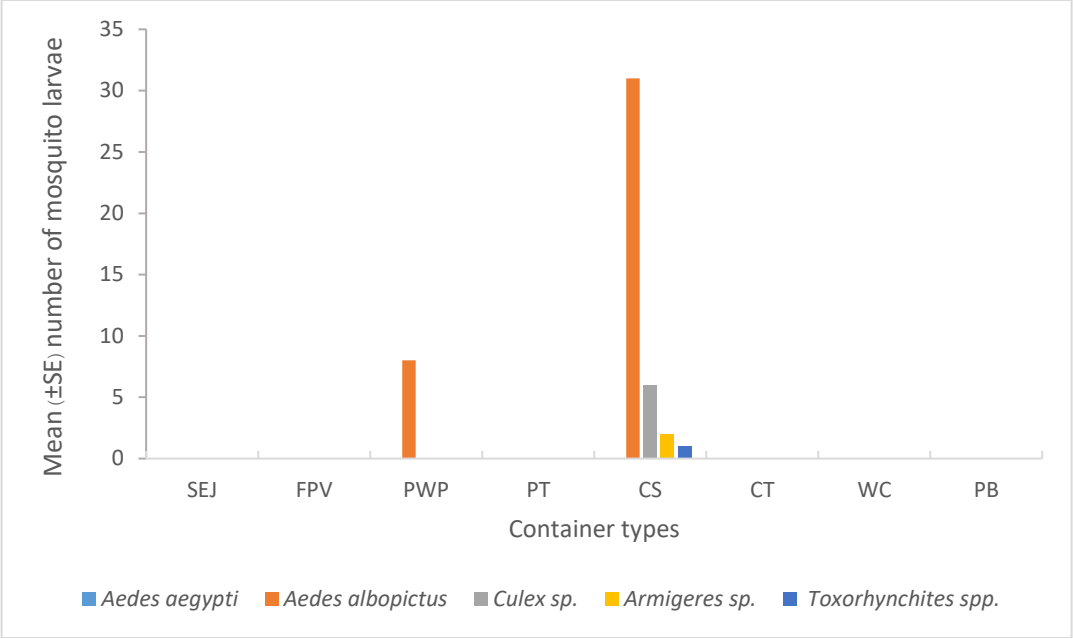


Figure 2 Percent number of containers were found in this study; (a) dormitory area and (b) academic area. FPV = flower plastic vases, FGV = flower glass vases, FCV = flower ceramic vases**,** PT = plastic tank, PWP = plant water pot, BO = bowls, CS = coconut shells, SEJ = small earthen jars, CT = cement tank, OT = others (artificial pond, bottle used, cup used, water bucket, natural pond by track of wheel, large earthen jar), PB = plastic bottles, PV = pottery vases and WS = waste containers

(a)



(b)

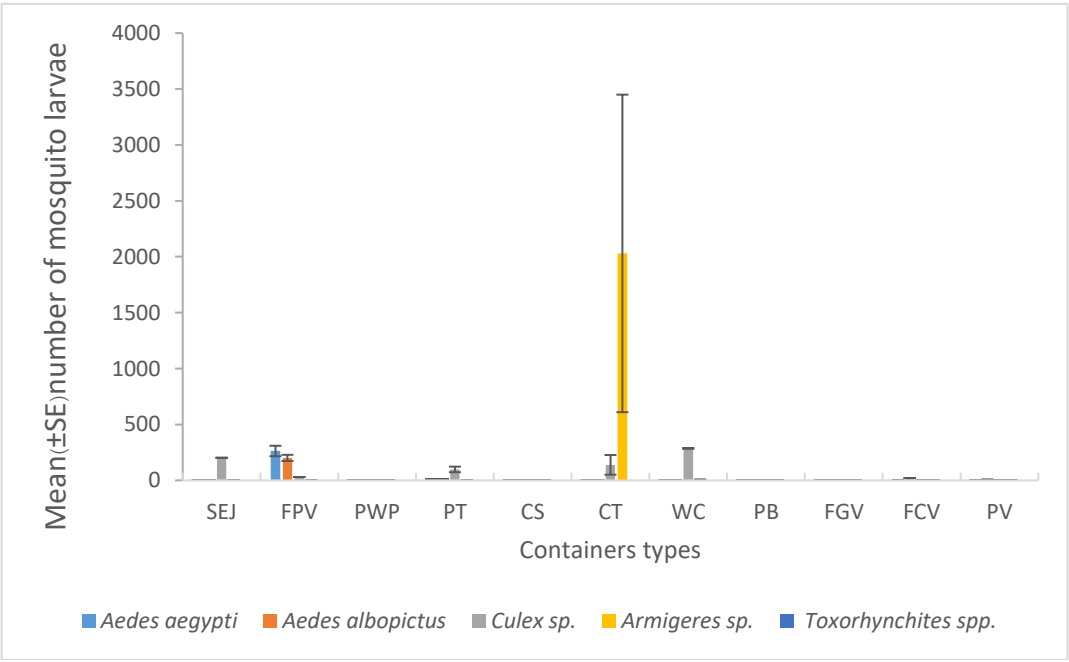


Figure 3 Different mosquito larvae species in different water containers; (a) dormitory area and (b) academic area. SEJ = small earthen jars, FPV = flower plastic vases, PWP = plant water pot, PT = plastic tank, CS = coconut shells, CT = cement tank, WC = waste container, PB = plastic bottles, FGV = flower glass vases, FCV = flower ceramic vase and PV = pottery vases

Table 1 Mosquito larvae species and number of mosquito larvae from two different workspaces in Suranaree University of Technology $\left({}^{*}P<0.05, {}^{**}P<0.01, {}^{***}\mathrm{P}< 0.001\right)$.

Work space	Mosquito larvae species and number of mosquito larvae					Statistical test
	Aedes aegypti	*Aedes albopictus*	*Culex* sp.	*Armigeres* sp.	*Toxorhynchites* spp.	
Dormitory	-	39	6	2	1	$X_3^2 = 82.16667^{***}$
Academic	272	244	755	2,041	1	$X_4^2 = 4035.667^{***}$

Table 2 The number of households and container, and larvae indices in dormitory area and academic area

	Dormitory area					**Academic area**				
	Aedes aegypti	***Aedes albopictus***	***Culex* sp.**	***Armigeres* sp.**	***Toxorhynchites* spp.**	***Aedes aegypti***	***Aedes albopictus***	***Culex* sp.**	***Armigeres* sp.**	***Toxorhynchites* spp.**
No. of households	13	13	13	13	13	17	17	17	17	17
No. of positive households	0	4	1	1	1	8	7	7	4	1
No. of containers	548	548	548	548	548	640	640	640	640	640
No. of positive containers	0	28	1	1	1	24	11	16	12	1
Larval index										
House Index (HI%)	0	30.77	7.69	7.69	7.69	47.06	41.18	41.18	23.53	5.88
Container Index (CI%)	0	5.11	0.18	0.18	0.18	3.75	1.72	2.50	1.88	0.16
Breteau Index(BI)	0	215.38	7.69	7.69	7.69	141.18	64.71	94.12	70.59	5.88

Discussion:

This is the first comprehensive study on the occurrence, abundance of mosquito species in Suranaree University of Technology at both academic and dormitory area, where the students spend most of their time there.

We found that, two positive containers within the dormitory area (i.e. plant water pot), contained four type of mosquito larvae (*Ae. albopictus*, *Culex* sp., *Armigeres* sp., and *Toxorhynchites* spp.) and 12 positive containers at academic area (i.e. flower plastic vases, plastic tanks, others (e.g. artificial pond, bottle used, cup used, water bucket, natural pond by track of wheel, large earthen jar), cement tanks, small earthen jars, flower glass vases, plant water pots, pottery vases, flower ceramic vases, paint buckets, wastes containers and bowls), contained all five types of mosquito larvae (*Ae. aegypti*, *Ae. albopictus*, *Culex* sp., *Armigeres* sp., and *Toxorhynchites* spp.). *Aedes* spp. larvae were found mostly in flower plastic vases that agree with the another study that were found *Aedes* mosquitoes can breed in variety of natural and man-made containers, such as water storage jars, cement tanks, other water jars, plastic tanks, flower vases, animal feeders, ant traps, and trash, such as glass, plastic bottles, cans, coconut husks and tires (Thavara et al, 2001; Chareonviriyaphap et al, 2003). Another study observed that, *Armigeres* sp. was identified as waste water restricted species in Swat Ranizai, Malakand province, Pakistan, previous study was found this mosquito species in bamboo glasses and discarded containers (Ali et al., 2013; Naz et al., 2014). Our results supported the previous works that *Armigeres* sp. larvae were found in waste containers, small earthen jars, cement tanks, plastic tanks, flower plastic vases and coconut shells. Due to this study, *Culex* sp. larvae were found in waste containers, small earthen jars, cement tanks, plastics tanks which were supported by Hribar et al. (2001) which reported that *Culex* sp. larvae are found in several water container types including ceramic vessels, plastic vessels, metal vessels, plastic water barrels, metal water barrels and concrete water tanks with most larvae in metal vessels. Another study studied *Toxorhynchites spp.* larvae was found in natural and artificial water containers such as bamboo stump, clay pots, broken bottles (Say, 1823; Nyamah et al., 2011; Odo et al., 2015) that related to our study which found *Toxorhynchites spp* larvae in coconut shell and small earthen jar.

The larval indices for both dormitory and academic area in the study were calculated to indicate of prevalence of vector in each area. We observed that HI in both of area for *Aedes* spp. were >5%, that is higher than WHO standard, so indicate our study area are dengue sensitive.

Conclusion: Our results are the first report shown that *Ae. albopictus*, *Culex* sp., *Armigeres* sp. and *Toxorhynchites* spp. larvae were found in both dormitory and academic area, *Ae. aeqypti* was found only in academic area. *Armigeres* sp. was found in the highest number in academic area. The breeding sites of mosquito larvae were different container types, *Armigeres* sp. was found mostly in cement tank, *Ae. aegypti* and *Ae. albipictus* were found mostly in flower plastic vase, *Culex* sp. was found mostly in waste containers and *Toxorhynchites* spp. was found in small earthern jars, coconut shells. All three larval indices showed that all workspaces have House Index (HI) for *Aedes* spp. was >5 %, Container Index (CI) for *Aedes* spp. was >5% and Breteua Index (BI) for *Aedes* spp. was >20 that indicates that at SUT either dormitory or academic area are dengue sensitive. Further research should be conducted to investigate the prevalence of different types of mosquito larvae in other schools in Nakhon Ratchasima.

In addition, larval control (source reduction or suppression) has been identified as one of the most effective methods for the control of mosquito borne diseases (Singh et al., 2006), we should monitor and share our mosquito larvae to other schools or community to develop the effective strategy to decrease the number of mosquito larvae and DHF incident cases in Thailand.

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References:

Adelman ZN, Jasinskiene N and James AA. Development and applications of transgenesis in the yellow fever mosquito, *Aedes aegypti*. *Mole. Bio. Par*. 2002; 121(1): 1-10.

Ali N, Marjan, Khalid K and Kausar A. Study on Mosquitoes of Swat Ranizai Sub Division of Malakand. *Pakistan J. Zool*. 2013; 45(2): 503-510.

Chareonviriyaphap T, Akratanakul P, Nettanomsak S, and Huntamai S. Larval habitats and distribution patterns of *Aedes aegypti* (Linnaeus) and *Aedes albopictus* (Skuse), in Thailand. *Southeast Asian J. Trop. Med. Public Health*. 2003; 34: 529-35.

Chumsri A, Tina F, Jaroensutasinee M, Jaroensutasinee K and Sririsathitkul Y. *Aedes*, *Culex* and *Mansonia* spp. Mosquito larval prevalences in Nakhon Si Thammarat, Thailand. *Proceeding of the* 41^{th} *congress on Science and Technology of Thailand*. 2015: 447-455.

Department of disease control. Dengue Hemorrhagic Fever (DHF) follow-up report week 53 Year 2017, 2018:1-2.

Department of disease control. Prediction of Dengue Hemorrhagic Fever (DHF) Year 2018, 2018:4-5.

Giatropoulos A, Emmanouel N, Koliopoulos G, and Michaelakis A. A Study on Distribution and Seasonal Abundance of *Aedes albopictus* (Diptera: Culicidae) Population in Athens, Greece. *J. Med. Ent*. 2012; 49(2): 262-269.

GLOBE Hydrology protocols, "Mosquito larvae", www.globe.gov. 2017.

Gopalakrishnan R, Baruah I and Veer V. Monitoring of malaria, Japanese encephalitis and filariasis vectors, *Med. J. Arm. F. India*. 2017; 70:129-133.

Hribar LJ, Smith JM, Vlach JJ and Verna TN. Survey of container breeding mosquitoes from the Florida Keys, Monroe County, Florida. *J. Am. Mosq. Contr. Assoc.* 2001; 17: 245-248.

Naz R, Maryam A, and Shabnam .Population Dynamics of Mosquitoes in Various Breeding Habitats at University of Peshawar Campus, Khyber Pukhtunkhwa Pakistan. *J. Ent. Zoo. Stu.* 2014; 2 (2): 189-195.

Nikookar SH, Fazeli-Dinan M, Azari-Hamidian S, Mousavinasab SN, Aarabi M, Ziapour SP, et al. (2017) Correlation between mosquito larval density and their habitat physicochemical characteristics in Mazandaran Province, northern Iran. *PLoS Negl Trop Dis* 11(8): e0005835.

Nyamah MA, Sulaiman S. and Omar B. Field observation on the efficacy of *Toxorhynchites splendens* (Wiedemann) as a biocontrol agent against *Aedes albopictus* (Skuse) larvae in a cemetery. *Trop*. *Bio*. 2011; 28(2): 312-319.

Odo GE, Agwu EJ, and Haruna AS. Culicid forms distribution and breeding sites in Nsukka ecological zone of South Eastern Nigeria. *J. Par. Vec. Bio*. 2015; 7(5): 94-100.

Preechaporn W, Jaroensutasinee M, Jaroensutasinee K, and Saeton J. Seasonal and Topographical Factors Affecting Breeding Sites of *Culex* Larvae in Nakhon Si Thammarat, Thailand. *Walailak J Sci & Tech*. 2007; 4(1): 23-39.

Rattanarithikul R and Panthusiri P. Illustrated Keys to the Mosquitoes of Thailand II. Genera *Culex*. *Southeast Asian J. Trop. Med. Public Health*. 1994; 25 (suppl 1): 1-66.

Rattanarithikul R, Harbach RE, Harrison BA, Panthusiri P, Jones JW, and Coleman RE. Illustrated Keys to the Mosquitoes of Thailand II. Genera *Culex*. *Southeast Asian J Trop Med Public Health* . 2005; 36 (suppl 2): 3-4.

Rueda LM. Global diversity of mosquitoes (Insecta: Diptera: Culicidae) in freshwater. *Hydrobiology*. 2008; 595: 477-487.

Saleeza SNR, Norma-Rashid Y, and Sofian Azirun M. Mosquito species and outdoor breeding places in residential area in Malaysia. *Southeast Asian J. Trop. Med. Public Health*. 2013; 44(6): 963-969.

Service MW. Medical entomology for student. 2^{nd} ed. Cambridge: University Press, 2000.

Shi XZ, Kang CJ, Wang SJ, Zhong X, Beerntsen BT, and Yu XQ. Functions of *Armigeres subalbatus* C-type lectins in innate immunity. *Ins. Bio. Molec. Bio*. 2014; 52: 102-114.

Singh RK, Dhiman RC and. Mittal PK. Mosquito larvicidal properties of Momordica charantia Linn (Family: Cucurbitaceae) .*J. Vect. Borne Dis*. 2006; 43: 88–91.

Strickman D and Kittayapong P. Dengue and its vectors in Thailand: calculated transmission risk from total pupal counts of *Aedes aegypti* and association of wing-length measurements with aspects of the larval habitat. *American J. Trop. Med. Hyg*. 2003; 68: 209–217.

Thangamathi P, Ananth S, Kala N, Maheshwari R, Gnanasoundrai A, and Nagamani N. Seasonal variations and physicochemical characteristics of the habitats in relation to the density of dengue vector *Aedes aegypti* in Thanjavur, Tamil Nadu, India. *Int. J. Sci. Nat*. 2014; 5(2): 271-276.

Thavara U, Tawatsin A, Chansang C, Kong-ngamsuk W, Paosriwong S, Boon-Long J, Rongsriyam Y, and Komalamisra N. Larval occurrence, oviposition behavior and biting activity of potential mosquito vectors of dengue on Samui Island, Thailand. *J. Vect. Eco*. 2001: 172-180.