Container types and water qualities affecting on number of *Aedes* larvae in Trang province, Thailand

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Abstract

This study investigated the effects of container types, and water qualities on numbers of *Aedes* mosquito larvae. Mosquito larvae were collected from nine sub-districts with ten households per sub-district in Trang province in March 2017 (i.e. Nongtrut, Natoming, Khuanpring, Bangrak, Khoklo, Banpho, Namphud, Tubtheang, and Nayongtai districts). We collected mosquito larvae in water containers using fishnets (0.55 mm mesh size) and preserved the mosquito larvae in 70% ethanol in the laboratory. We grouped containers in three groups (jar and tank, dustbins and others such as areca nut preservation jars and animal food bowls). We recorded water qualities in each container (presence of vegetation and algae, water level, odour, turbidity, temperature and pH). We identified the mosquito larvae to genus level and counted the numbers. We calculated the mosquito larvae indices (House index (HI), (Breteau index (BI), and Container index (CI)). For this study, we focused only on *Aedes* species; other species were discarded. We observed that container types, presence of algae in water, water odour, temperature and pH did not have effects on *Aedes* species larval numbers. Larval numbers were higher in clear and without vegetation water than in turbid and vegetative water, respectively. In addition, 50-75% of water level in the containers was the most preferred level for the *Aedes* species. Among nine sub-districts, Khoklo, Banpho, and Tubthiang had the highest HI, and Tubthiang had the highest BI and CI.

Keywords: Water containers, *Aedes* species, water qualities, mosquito larval index

Introduction

Thailand is suffering from one of the highest rates of dengue fever (DF) and dengue hemorrhagic fever (DHF) in the world [1]. In southern Thailand, mosquito borne disease is one of the major problems [2-3]. Dengue cases in southern Thailand from 2010-2016 were 31192, 5668, 11119, 24629, 12095, 11925 and 17169 cases per year, respectively. From January-November 2017, there were a total of 11340 dengue cases reported. In Trang province, the dengue incidences from 2011-2016 were 286, 679, 1120, 264, 488 and 748, respectively. From January-November 2017, there were 295 dengue cases reported [4].

Dengue fever is caused by dengue viruses in the family *Flaviviridae*, transmitted principally by *female* Ae. aegypti and possibly by Ae. albopictus in the tropical and sub-tropical regions of the world [5]. *Aedes* mosquitoes also transmit chikungunya, yellow fever and Zika infections. Dengue vaccine has been discovered to prevent dengue fever in humans but World Health Organization only recommends the vaccine as a possible option in areas of the world where the disease is common [6]. Larval control (source reduction or suppression) is still one of the most effective methods for controlling of mosquito borne diseases [7]. Containers are probably the most important factor determining the breeding of different mosquito species [8]. Different kinds of containers are used as mosquito breeding sites [2-3,9,10]. *Aedes* mosquitoes can be found inside and outside of the houses [9,11]. In Thailand, most households store water in various types of water containers for cooking and bathing, and that is why *Aedes* mosquitoes are important threat for DHF [12]. Mosquito oviposition behaviour is affected by various physico-chemical properties of the water [13].

In Thailand, limited studies have investigated the effects of water qualities on mosquito larval distribution. Most of the studies have focused on larval surveillance only [14-17] without testing the water qualities. Therefore, conducting research on this topic is very important. In this study, we investigated the effects of container types and water qualities on the *Aedes* mosquito larvae in Trang province, Thailand.

Materials and Methods

Study site

The study site was located at Muang Trang, southern Thailand (7° 33' 22.79" N and 99° 36' 41.08" E) (Figure 1), and research was conducted in March 2017. This district is about 533 km^2 and is composed of 15 sub-districts, 119 villages, 21,012 houses, 155,441 populations and the density of 291.63 people/km^2 (Muang Trang District Register Office).

Mosquito larvae collection, preservation and identification

We categorised nine sub-districts into three categories based on dengue risk area: (1) dengue high risk area (Nongtrut, Natoming and Khuanpring), (2) dengue moderate risk area (Bangrak, Khoklo and Banpho) and (3) dengue low risk area (Namphud, Tubthiang and Nayongtai). We collected mosquito larvae from all water containers in and around houses from randomly selected ten households per sub-district with a total of 90 households.

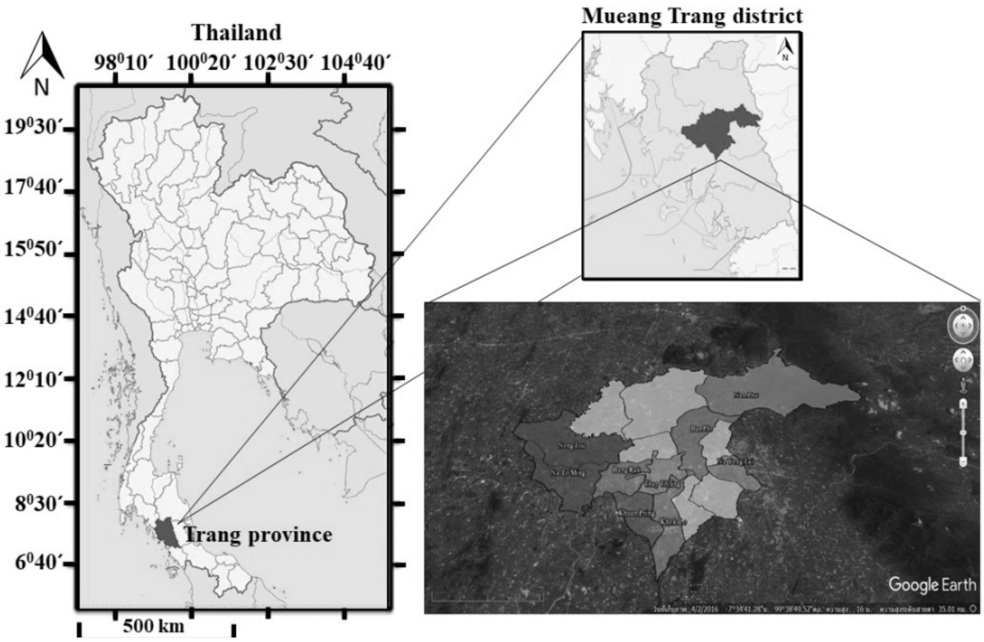


Figure 1 Location of the study site in Muang Trang province, Thailand.

During the larval survey, container types and water quality parameters (pH and temperature) were recorded as follows:

(1) type of water containers were classified into three groups: (a) water storage containers (earthen jars, cement tanks, plastic tanks), (b) dustbins (discard tires, used pots, used plastic glasses, coconut shells) and (c) others (areca nut preservation jars and animal food bowls).

(2) vegetation in water was classified into two groups: (a) with vegetation and *(*b*)* without vegetation

(3) water levels was classified into four water levels: (a) 0-25%, (b) 25-50%, (c) 50-75% and (d) 75-100%.

(4) turbidity was classified into two groups: (a) clear and (b) turbid.

(5) odour was classified into two groups: (a) no odour and (b) sewage odour.

(6) algae in water was classified into two groups: (a) with algae and *(*b*)* without algae.

(7) the size of the containers were divided into three groups based on perimeter of the containers: (a) less than 1 meter, (b) 1-10 meter, and (c) more than 10 meter.

(8) shaded area (shades provided by trees or man-made structures) was classified into five groups: (a) 0% (no shade), (b) 25% shade, (c) 50% shade, (d) 75% shade, and (e) 100% shade

Mosquito larvae were collected using fishnets of 0.55 mm mesh size in March 2017, placed in plastic bag, tied bags with rubber bands [18], brought back to the laboratory and preserved in 70% ethanol [19]**.** Each mosquito larva was identified to a species level under a microscope by using Rattanarithikul and Panthusiri's keys [20]. For this study, we focused only on *Aedes* species; other species were discarded. In Namphud, we did not find any mosquito larva.

Three larval indices (i.e., house index (HI), container index (CI), and Breteau index (BI)) were calculated, as per standard WHO guidelines (WHO, 2009). House index (HI) was calculated as the number of houses infested divided by the number of houses surveyed × 100. Container index (CI) was calculated as the number of positive containers divided by the number of containers surveyed × 100. Breteau Index (BI) was calculated as the number of positive containers divided by the number of houses surveyed × 100. HI is widely used to calculate the presence and distribution of *Aedes* species populations in a given locality. BI and HI are commonly used for the determination of risk areas for control measures to be implemented in. Generally, either HI > 5% or BI > 20% for any locality indicates that the locality is dengue sensitive [21].

Data analysis

We used independent sampled t-test to test the mean differences in the number of mosquito larvae between water containers- (i) with and without vegetation (ii) water containers with different turbidity (clear and turbid), (iii) water containers with different odour, (iv) with and without algae (v) water body, and One way ANOVA test was used to test the differences in the number of mosquito larvae among: (i) different kinds of water contains (ii) water containers with different levels of water, and (iii) different kinds of shade. Correlations were used to test the associations between (1) the number of *Aedes* larvae and temperature and (2) the number of *Aedes* larvae and pH. The significant tests were two-tailed with a significant level at $P<0.05$.

Results

Aedes larvae and sub-districts

In Thapthiang, Bangrak, Khoklo, and Nayongtai sub-districts, the number of *Ae.aegypti* larvae was higher than *Ae.albopictus.* On the other hand, in Banpho, the number of *Ae. albopictus* larvae were higher than *Ae.aegypti*. The number of *Aedes* larvae in moderate and low risk area were higher than high risk area. The numbers of Aedes larvae among sub-districts in each risk area did not differ but the total larvae numbers were different among high, moderate and low risk areas (Table 1).

***Aedes* larvae and water qualities**

Aedes larvae were more in numbers in water with no vegetation, 50-75% water level, clear water than in water with vegetation and turbid water (Figure 2a-c). Other factors (i.e. container groups, algae in water, water odour, water body, and shaded area did not have any effects on the number of *Aedes* larvae (Figure 2d-h).

The number of *Aedes* larvae was not associated with water temperature and pH (temperature: R = -0.13, *ns*; pH: R = -0.14, *ns*).

Mosquito indices at Muang Trang, Thailand

Among nine sub-districts, Khoklo, Banpho, and Tubthiang sub-districts had the highest HI, and Tubthiang sub-district had the highest BI and CI.

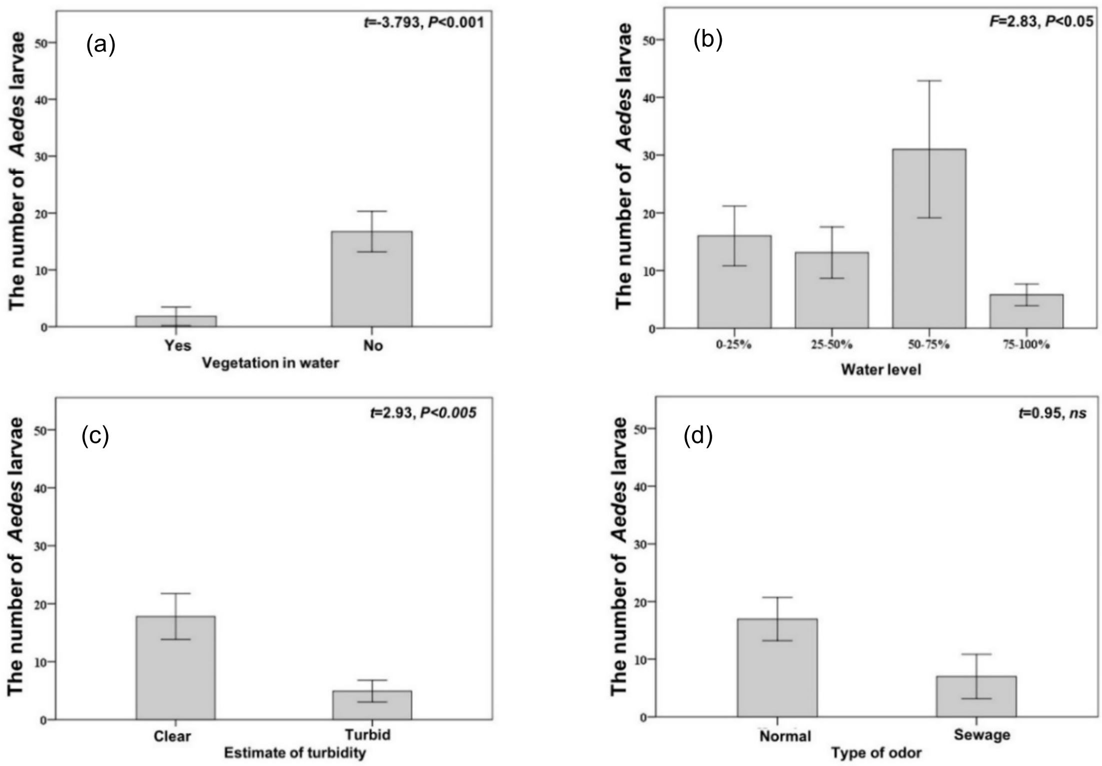
Table 1 The number (mean ± SE) of *Ae. aegypti* and *Ae. albopictus* larvae in Trang, southern Thailand (*P < 0.05).

Dengue Risk area	Sub-district	*Aedes* larvae in each sub-district	Total *Aedes* larvae in each risk area	Statistical tests	
				One-way ANOVA among sub-districts	One-way ANOVA among risk areas
High	Natoming	2.00±5.26			
	Nongtrud	2.86±7.14	2.22±0.96[a]	$F_{2,29}$=0.180, *ns*	
	Khunpring	1.50±2.32			
Moderate	Bangrak	8.81±16.45			
	Khokloa	3.35±2.91	8.11±2.33[b]	$F_{2,51}$=1.475, *ns*	$F_{2,121}$=3.201*
	Banpho	12.78±24.89			
Low	Namphud	0.00±0.00			
	Tubthiang	15.43±23.66	12.39±3.53[b]	$F_{2,35}$=1.420, *ns*	
	Nayongtai	1.33±1.75			

Remark: a, b, and c letters represent the mean differences (P<0.05) in *Aedes* larvae numbers among three risk areas.

Table 2 Positive houses, positive containers, and *Aedes* mosquito larval indices in nine sub-districts in Trang province, southern Thailand.

Parameters	Dengue Risk Area								
	High Risk Area			Moderate Risk Area			Low Risk Area		
	Natoming	***Nongtrut***	***Khuanpring***	***Bangrak***	***Khoklo***	***Banpho***	***Namphud***	***Tubtheang***	***Nayongtai***
No. of positive households	3	1	2	3	6	6	0	6	3
No. of containers	47	51	39	64	44	40	62	56	47
No. of positive containers	3	3	4	4	10	6	0	14	3
Larval index									
HI (%)	30.00	10.00	20.00	30.00	60.00	60.00	0.00	60.00	30.00
CI (%)	6.38	5.88	10.26	6.25	22.73	15.00	0.00	25.00	6.38
BI	30.00	30.00	40.00	40.00	100.00	60.00	0.00	140.00	30.00



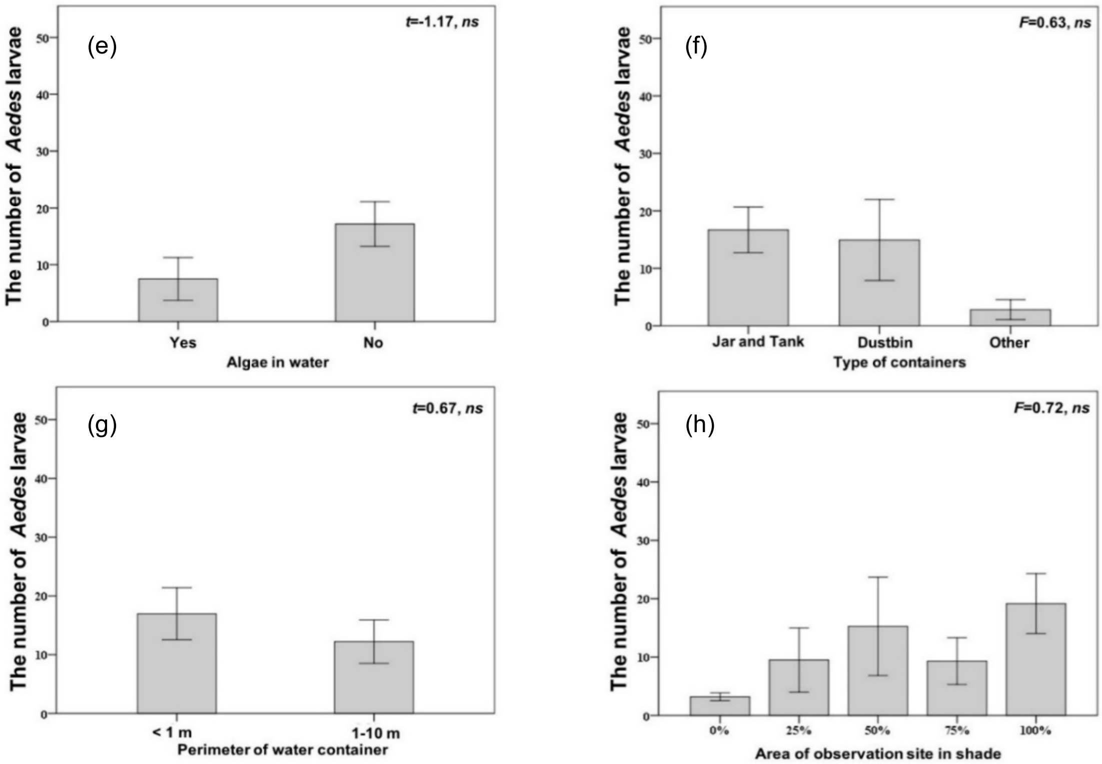


Figure 2 The number of mosquitoes in different conditions and (a) vegetation in water (b) water level (c) algae in water, (d) turbidity, (e) odour type, (f) container types, (g) water body, and (h) shaded area.

Discussion

The number of *Aedes* larvae was higher in water without vegetation than water with vegetation. It indicates that *Aedes* species prefers clear water more than vegetative water, however they can survive in water with vegetation. According to Dom *et al*. [22], *Aedes* species can thrive in a variety of water such as clear, turbid or polluted (contaminated water due to toxic chemicals or fertilizers) water. Another study observed that *Ae. aegypti* had a negative association with waters covered by canopy and mixed vegetation [*23*].

The number of *Aedes* larval was highest in 50-70% water level. Dieng *et al.* [24] observed that *Ae. albopictus* females preferred to lay eggs in containers that had 50% water levels. The question is why the numbers of mosquito larvae were very low when the containers were almost full with water? The reason could be that, during rainfall, the containers become full with water very rapidly that has a detrimental effect on mosquito larvae as the larvae have high chances to be splashed out from the containers. That might be why the numbers of *Aedes* mosquito were lower when the containers were almost full of water.

The larval indices in nine sub-districts indicate the risk of dengue fever in sub-districts. We observed that Khoklo, Banpho, and Tubthiang sub-districts had the highest HI, and Tubthiang sub-district had the highest BI and CI than other sub-districts. According to WHO, an area would be classified as a high dengue risk area when it has HI> 10%, and low risk when it has HI <1%. In our study, in all sub-districts (except Namphud), House Index (HI) was >10%, which indicates that all sub-districts except Namphud are classified as dengue high risk areas. Among all sub-districts, Tubthiang sub-district is the highest dengue risk area as it has the highest HI, BI, and CI indices. Similarly, Preechaporn *et al*. [9] reported that HI for *Aedes* species was higher than WHO standard in three topographical areas (mangrove, rice paddy and mountain) in Nakhon Si Thammarat, southern Thailand, that indicated the high risk of dengue in those areas.

Conclusions

We observed that container groups, algae in water, water odour, water body, shaded area, water temperature and pH did not have any effect on the number of *Aedes* larvae. The number of *Aedes* larvae was higher in water without vegetation than water with vegetation. All sub-districts (except Namphud) have higher Hi, BI, and CI based on the WHO standard, which indicates that all sub-districts (except Namphud) are high dengue risk areas. Local community should launch some campaigns to raise some awareness on dengue fever in these areas.

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