



Larval breeding characteristics and distribution of *Aedes* mosquito species in the economic capital of Benin: A public health concern

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Abstract

Aedes mosquito is a vector for transmitting numerous arboviroses. Knowledge of the breeding habitat of this mosquito is crucial for preventing arboviroses occurrence and for implementing appropriate interventions for control. Therefore, this study aims to assess the breeding habitats and presence of *Aedes* mosquito species in the study areas.

A house-to-house cross-sectional entomological survey was carried out in three localities of Cotonou, the economic capital of Benin in order to investigate the presence of larval breeding sites of *Aedes* mosquito. All available water-holding containers found in and around houses were inspected for the presence of immature stages of *Aedes* mosquitoes, and they were collected and reared to the adult stage for species identification.

Of the 450 houses surveyed for the presence of *Aedes* mosquito larval breeding. About 15.77% of a total of 479 water-holding inspected containers were found to be infested with *Aedes* mosquito larva. Discarded tires had an especially high positivity rate for *Aedes* mosquito larvae (54.93%) and this is perfectly explained by the marketing of second-hand tires in the study area. The dominant *Aedes* mosquito species that emerged from the collected larvae was *Ae. Aegypti*, suggesting a potential threat for arbovirus transmission.

This study highlights the need for the establishment of a suitable entomological surveillance program for *Aedes* mosquito. Additionally, public health education, creating knowledge and awareness of the residents on mosquito-borne diseases should be advised.

Keywords: *Aedes* mosquito, breeding habitat, threat, Benin

1. Introduction

Aedes mosquito is the main vector for the transmission of numerous mosquito-borne viruses (arbovirus) such as Zika virus, dengue virus, yellow fever virus and chikungunya virus [1-4]. The global burden of these diseases has significantly increased in several countries in the world during the past decades [5-8]. Zika was declared a public health emergency of international concern in February 2016 [9]. The spread of these viruses follows the presence of the primary vector, *Aedes aegypti* [10] which was formerly found in sub-Saharan African where it originated [11], but has now spread to other continents through trade spread and currently is distributed worldwide through man-made activities [12,13].

Aedes aegypti mosquito is distributed in urban areas and breeds in indoor and outdoor settings in a wide variety of natural and artificial water-holding containers such as rubber tires, plastic tanks, water storage jars, cement tanks, flower vases, plastic bottles. Moreover, several climate and environmental conditions such as weather variables (rainfall, relative humidity and temperature) may play an important role in *Aedes* mosquito abundance and distribution [14].

Several biotic and abiotic factors might enhance the risk of transmission of *Aedes* -borne arboviral diseases in Benin. In

fact, the long periods of dry season in some parts of the country have increased leading people to opt for water storage practices which might increase the availability of breeding habitats of *Aedes* larvae. In addition, the marketing of second-hand tires from Europe, America and Asia remains a lucrative activity in Cotonou, the economic capital city of Benin. The storage of these second-hand tires offers good breeding sites for *Ae. aegypti* in this area. The unplanned urbanization in Benin still also remains wide, favoring the presence of high population densities with associated artificial breeding sites for *Aedes* mosquitoes.

Very little information is available on *Aedes* mosquito primary breeding sites and on the efficiency of *Aedes* mosquito control measure in Benin. In this study, we report through Globe Observer (a platform developed by Globe Zika project for *Aedes* larvae breeding identification) on the first more elaborate entomological *Aedes* surveys in Benin. This study provides therefore baseline information on the types of Zika vectors breeding in natural and artificial water storage materials. The results of this study will enable providing community awareness about *Aedes* mosquitoes and the preventive and control interventions to be implemented.

2. Material and Methods

Study Area

The study was conducted in Cotonou (Figure 1). Cotonou is a municipality and the economic capital of Benin located in the southern part of the country. It is located at 6°21'55''N

and 2°25'05''E. The main landscape characteristic of this municipality is the urban area with low urbanization. The major land area in this study area is commercial, residential, and industrial area.

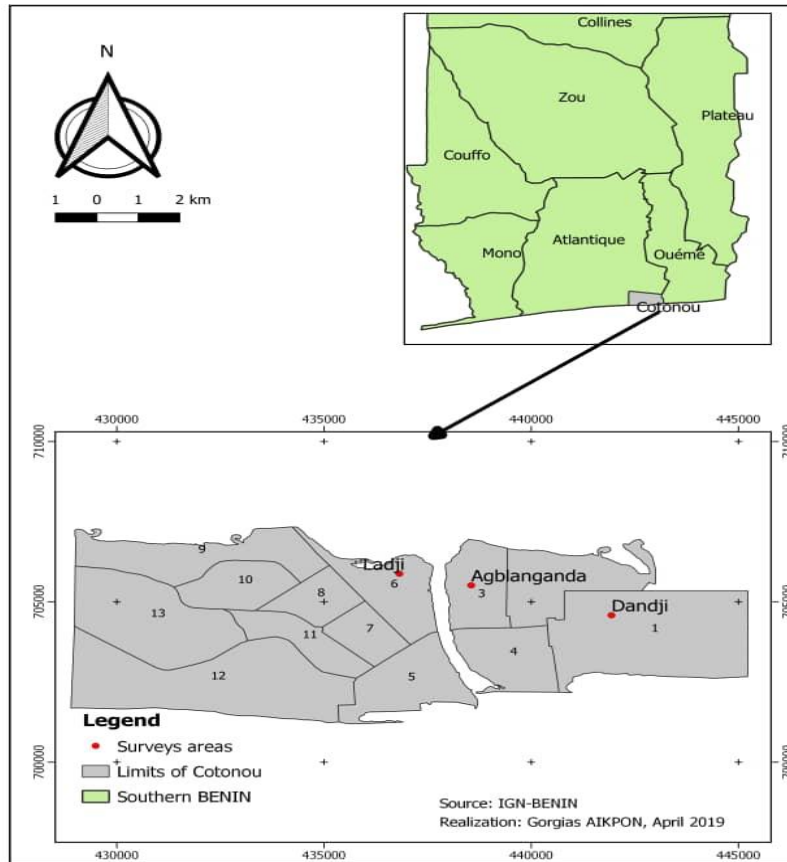


Fig 1: Map of study area.

Data collection

The cross-sectional study was conducted between March and April 2019 during the rainy season. A house-to-house entomology survey was carried out in houses and peridomestic areas to detect mosquito larval breeding sites in three localities (Agblangandan, Dadji and Ladji) in the municipality of Cotonou. A total of 450 households (150 in each locality) were included in the study.

Therefore, all containers (Figure 2) both indoors and outdoors which might harbor mosquito larvae and pupae were inspected to check the presence or absence of mosquito larvae and pupae. The number and type of containers inspected were recorded, including information on which had or did not have immature stages of mosquitoes. Larvae and pupae presumed to be *Aedes* mosquitoes were collected using a plastic cup and pipette. The entire contents of the various containers were emptied into a large plastic tray and the larvae and pupae specimens were picked out using a dropper. All collected immature specimens were reared in the laboratory until adult emerged. All adults that emerged from pupae were collected and stored in vials and identified at species level using microscope and identification keys [15-16].

Moreover, positive larval breeding sites (Which containing larvae) have been geo-referenced and the data is recorded on the Globe observer platform. This platform developed by « Globe Zika Program » helps to identify *Aedes* mosquito

larvae breeding sites and make the public aware of the risk of having larval breeding sites on the premises, and to provide information on ways to dispose of or prevent breeding sites.

Data analysis

The larval survey data were calculated and analyzed in terms of larval indices. Therefore three larval indices: House Index (HI), Container index (CI) and Breteau Index (BI) were worked out as stated in WHO guidelines. Breeding preference ratio was also calculated through container preference of *Aedes* larval breeding [17].

$$HI = \frac{\text{Number of houses infested}}{\text{Total number of houses inspected}} \times 100$$

$$CI = \frac{\text{Number of positive containers}}{\text{Total number of containers inspected}} \times 100$$

$$BI = \frac{\text{Number of positive containers}}{\text{Total number of houses inspected}} \times 100$$

3. Results

Potential larval breeding sites of *Aedes* mosquito

Overall, a total of 450 houses (both outside and inside) were

visited during the entomological surveys in the study area to detect the presence of *Aedes* mosquito breeding sites. A total of 479 water-holding containers were inspected (figure 2), of which 71 (15.77%) were found to be infested with *Aedes* mosquito larva. Figures 3 and 4 show the kind of

water-holding container found in the study area with the highest rate of positivity for *Aedes* mosquito larvae was discarded tires (54.93%), followed by plastic containers (15.49%), cement barrel for water storage (12.68%), ditch (9.86%), Mud pot (7.04%).

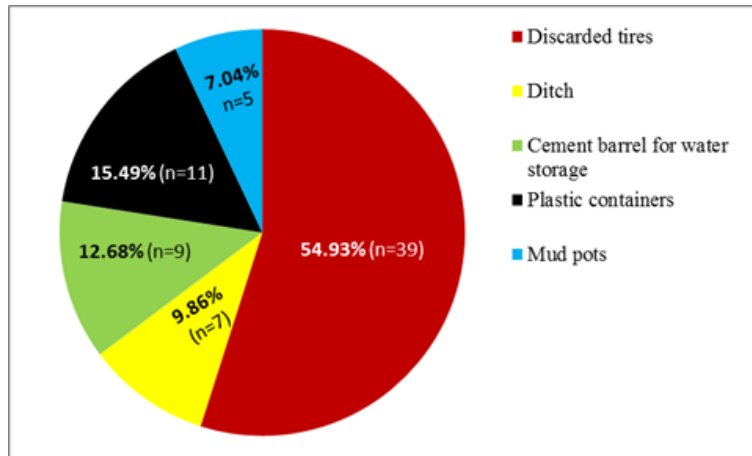


Fig 2: Type of containers inspected for *Aedes* mosquito larval breeding in the study area.



Fig 3: Main *Aedes* mosquito breeding habitats identified during a larval survey, (A, C) Cement barrel for water storage; (B, D) discarded tires; (E) Stored tires for sell; (F) ditch.

***Aedes* mosquito larval indices**

Of the 450 houses surveyed, 68 had *Aedes* mosquito breeding habitats. The figure 4 shows details about houses and containers inspected for *Aedes* mosquito larval infestations in the study area. The proportion of infested houses by *Aedes* mosquito larvae (HI) was 15.11 in this

study. Overall, 479 water-holding containers were found, of which 71 had *Aedes* mosquito breeding. The Container Index was therefore 14.82. The Breteau Index, as number of positive containers per 100 houses inspected was recorded as 15.77.

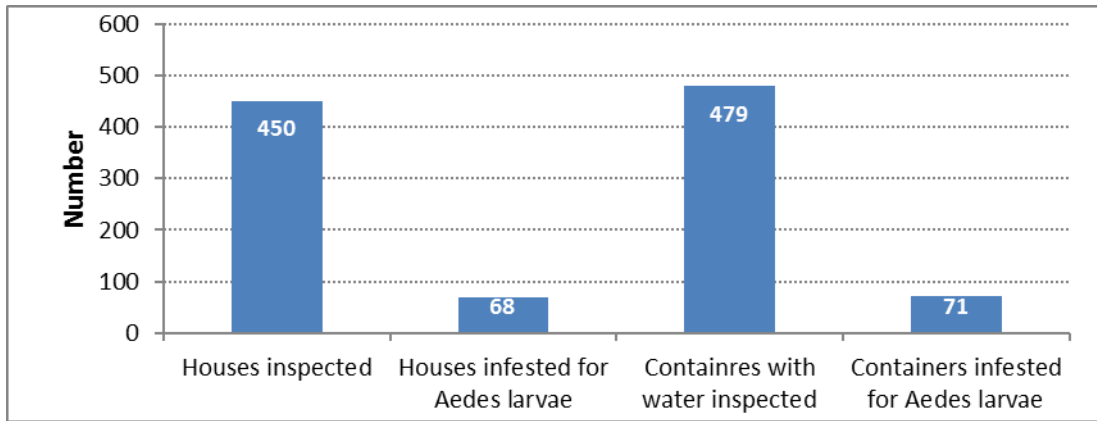


Fig 4: Houses and containers inspected for *Aedes* mosquito larval infestations in the study area.

Aedes species identification

Aedes larvae were collected in the positive containers and were transported in well labelled plastic bottles to the laboratory of the Centre de Recherche Entomologique de Cotonou, Benin (CREC) where they were maintained at 28 ± 2 C and 72 ± 5% relative humidity. Adult mosquitoes from larvae collections were morphologically identified to species using taxonomic keys [20, 21]. Overall, 1016 adult mosquitoes were identified. These

mosquitoes belonged to six species that are *Aedes aegypti*, *Aedes Vitatus*, *Aedes luteocephalus*, *Aedes longipalpis*, *Aedes gr. palpalis* and *Aedes gr. tarsalis* (figure 5). The two most abundant species collected were *Aedes aegypti* and *Aedes vitatus* with respectively 79.96% and 9.45% of the total collected. The other species (*Aedes luteocephalus*, *Aedes longipalpis*, *Aedes gr. palpalis* and *Aedes gr. tarsalis*) are poorly represented (less than 5% of the population).

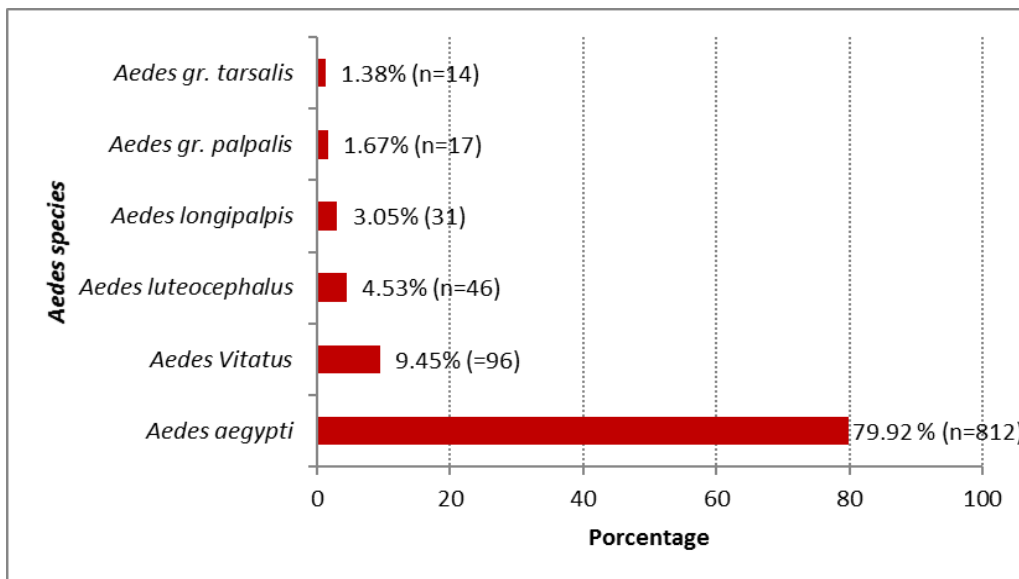


Fig 5: *Aedes* species diversity in the study area.

4. Discussion

The study was the first attempt to characterize the presence of *Aedes* mosquitoes and their preferred breeding habitats in Benin. The results of this study indicated most of the key breeding containers found in the study area were artificial or man-made containers and discarded tires had an especially high positivity rate for *Aedes* mosquitoes larvae. These findings concurred with previous studies done elsewhere [22-24]. This might have been because the weather conditions inside tires, such as cool temperature, humidity, and reduced light, which create a suitable environment for *Aedes* mosquito breeding [25-26]. The types of container, water quality, and conditions of water containers are important for breeding. Eggs stuck to the tires also play a role in the preservation of the *Aedes* mosquito population throughout the off season [27]. We also found other kinds of breeding containers included plastic containers, barrel for water

storage and these findings corroborated with previous studies in Malaysia where *Aedes* mosquitoes preferred all those key-breeding containers whether in urban, suburban or rural habitats [22, 28, 29].

The presence of water-holding containers allows *Aedes* mosquito larvae to breed, thereby increasing the *Aedes* mosquito population and the potential risk for arbovirus transmission. These findings might have important implications for *Aedes* mosquito control strategies, and in particular they may enable a more focused approach to vector control in which specific types of water-holding containers would be targeted. Specially, the tires trade, which is widely practiced in the study area, must be controlled and the tire storage shops must be covered to prevent rainwater from stagnating there to serve as breeding sites for *Aedes* mosquitoes. Moreover, vector control program could apply the entomological monitoring data to

reduce costs and make more efficient use of manpower and equipment by concentrating efforts on eliminating key *Aedes* breeding sites. Improved access of the communities to piped water supply will also reduce the need for water storage containers. Additionally and in the meantime, for the control of container breeding mosquitoes it is possible to use different methods in integration and these include covering water holding containers, public health education [30] like the Globe Zika project, creating knowledge and awareness of the residents on mosquito-borne diseases, and draining containers once a week.

This study shows that the dominant *Aedes* mosquito species that emerged from the collected larvae was *Ae. Aegypti*. This is consistent with the preference of *Ae. aegypti* females to lay their eggs in artificial containers [31]. *Aedes aegypti* is found in close proximity to human residences and therefore can feed easily on human blood. This increases the potential threat to the occurrence of arbovirus transmission among humans in this area. The second identified vector was *Ae. vittatus*, which is also a competent vector of arboviruses [32]. The current study presents few limitations that need to be highlighted. The study was carried out during the rainy season, which might have led to high values of the risk indicators. Moreover, this study involved only collection of mosquito larvae from discarded tires, household containers, and discarded water holding materials so that it needs further investigation to look for mosquito larvae in natural water holding containers and larger water tanks. Despite these limitations, this preliminary study provides the first baseline data and useful information on the presence of the arbovirus vectors *Aedes* mosquitoes in the study areas.

5. Conclusion

This study has provided valuable data on the characteristics and the distribution of *Aedes* mosquito species in a wide area of an urban district in Benin. The study establishes evidence for the presence of positive larval breeding sites of *Aedes* mosquitoes. Although we can note a diversity of *Aedes* larval habitats, the discarded tires are highly incriminated and this is perfectly explained by the marketing of the second-hand tires in the study area. The presence of *Aedes* mosquitoes especially *Aedes aegypti* species, represents a potential threat and it is clear that arbovirus transmission is more likely to occur in that area. Therefore, it will be important to implement urgently *Aedes* mosquito control strategies in order to prevent emergence of arboviral diseases such as Zika, dengue, yellow fever among others.

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7. References

1. Farraudière L, Sonor F, Crico S, Etienne M, Mousson L, Hamel R, *et al.* First detection of dengue and chikungunya viruses in natural populations of *Aedes aegypti* in Martinique during the 2013-2015 concomitant outbreak. *Rev Panam Salud Publica*, 2017, 41(e63).
2. Cigarroa-Toledo N, Blitvich B, Cetina-Trejo R, Talavera-Aguilar L, Baak-Baak C, Torres-Chablé O, *et al.* Chikungunya virus in febrile humans and *Aedes aegypti* mosquitoes, Yucatan, Mexico. *Emerg Infect Dis*, 2016, 22(10).
3. Ferreira-de-Brito A, Ribeiro I, de Miranda R, Fernandes R, Campos S, da Silva K, *et al.* First detection of natural infection of *Aedes aegypti* with Zika virus in Brazil and throughout South America. *Mem Inst Oswaldo Cruz*, Rio de Janeiro. 2016; 111(10):655-658.
4. Thonnon J, Fontenille D, Tall A, Diallo M, Renaudineau Y, Baudez B, *et al.* Re-emergence of Yellow Fever in Senegal in 1995. *Am J Trop Med Hyg.* 1998; 59(1):108-114.
5. Hennessey M, Fischer M, Staples JE. Zika Virus Spreads to New Areas-Region of the Americas, May 2015-January 2016. *MMWR Morb Mortal Wkly Rep.* 2016; 65(3):55-58.
6. Leroy EM, Nkoghe D, Ollomo B, Nze-Nkoghe C, Becquart P, Grard G, *et al.* Concurrent chikungunya and dengue virus infections during simultaneous outbreaks, Gabon, 2007. *Emerg Infect Dis.* 2009; 15(4):591-593.
7. Bhatt S, Gething PW, Brady OJ, Messina JP, Farlow AW, Moyes CL, *et al.* The global distribution and burden of dengue. *Nature.* 2013; 496(7446):504-507.
8. Nsoesie EO, Kraemer MU, Golding N, Pigott DM, Brady OJ, Moyes CL, *et al.* Global distribution and environmental suitability for chikungunya virus, 1952 to 2015. *Euro Surveill.* 2016, 21(20).
9. WHO. WHO statement on the first meeting of the International Health Regulations (2005) (IHR 2005) Emergency Committee on Zika virus and observed increase in neurological disorders and neonatal malformations 2016 July 23rd, 2017. Available from: <http://www.who.int/mediacentre/news/statements/2016/1st-emergency-committee-zika/en/>.
10. Kraemer MU, Sinka ME, Duda KA, Mylne AQ, Shearer FM, Barker CM, *et al.* The global distribution of the arbovirus vectors *Aedes aegypti* and *Ae. albopictus*. *Elife.* 2015; 4:e08347. <https://doi.org/10.7554/eLife.08347> PMID: 26126267; PubMed Central PMCID: PMC4493616.
11. Moore M, Sylla M, Goss L, Burugu M, Sang R, Kamau L, *et al.* Dual African origins of global *Aedes aegypti* s.l. populations revealed by mitochondrial DNA. *PLoS Negl Trop Dis*, 2013, 7(4).
12. Powell J, Tabachnick W. History of domestication and spread of *Aedes aegypti*- A Review. *Mem Inst Oswaldo Cruz*, Rio de Janeiro. 2013; 108(Suppl. I):11-17.
13. Kraemer M, Sinka M, Duda K, Mylne A, Shearer F, Barker C, *et al.* The global distribution of the arbovirus vectors *Aedes aegypti* and *Ae. albopictus*. *Ecol Epid Glob Health*, 2015.
14. Thammapalo S, Chongsuwatwong V, McNeil D, Geater A. The climatic factors influencing the occurrence of dengue hemorrhagic fever in Thailand. *Asian Journal of Tropical Medicine and Public Health.* 2005; 36:191-196.
15. Rueda LM. Pictorial keys for the identification of mosquitoes (Diptera: Culicidae) associated with dengue virus transmission. *Zootaxa.* 2004; 589:1-60.
16. Harrison BA. Field identification of adult and larval mosquitoes; [cited 2018 May 4]. Available from, 2005.

- <http://www.gamosquito.org/resources/fguideid.pdf>.
17. Kumar RR, Kamal S, Patnaik S, Sharma R. Breeding habitats and larval indices of *Aedes aegypti* (L) in residential areas of Rajahmundry town, Andhra Pradesh. The Journal of communicable diseases. 2002; 34(1):50-8.
 18. Webb CE. Mosquito ecology: field sampling methods. Aust Entomol. 2008; 47:382-383.
 19. World Health Organization. Dengue haemorrhagic fever: diagnosis, treatment, prevention and control, 2nd ed; [cited 2018 May 4]. Available from, 1997. <http://www.who.int/iris/handle/10665/41988>.
 20. Edwards FW. Mosquitoes of the Ethiopian Region. III. Culicine Adults and Pupae. London, UK. British Museum (Natural History), 1941.
 21. Rueda LM. Pictorial Keys for the Identification of Mosquitoes (Diptera: Culicidae) Associated with Dengue Virus Transmission. USA: Magnolia Press, 2004.
 22. Vijayakumar K, Sudheesh Kumar TK, Nujum ZT, Umarul F, Kuriakose A. A study on container breeding mosquitoes with special reference to *Aedes* (*Stegomyia*) *aegypti* and *Aedes albopictus* in Thiruvananthapuram district, India. J Vector Borne Dis. 2014; 51:27-32.
 23. Singh S, Rahman A. Contribution of *Aedes aegypti* breeding by different income group communities of Dehradun city, Uttarakhand, India. Biol Forum Int J. 2013; 5:96-99.
 24. Lloyd LS, Winch P, Ortega-Canto J, Kendall C. Results of a community-based *Aedes aegypti* control program in Merida, Yucatan, Mexico. Am J Trop Med Hyg. 1992; 46:635-642.
 25. Bi P, Zhang Y, Parton KA. Weather variables and Japanese encephalitis in the metropolitan area of Jinan city, China. J Infect. 2007; 55:551-556.
 26. Sripugdee S, Inmoung Y, Junggoth R. Impact of climate change on dengue hemorrhagic fever epidemics. Res J Appl Sci. 2010; 5:260-262.
 27. Rao BB, George B. Breeding patterns of *Aedes stegomyia albopictus* in periurban areas of Calicut, Kerala, India. Southeast Asian J Trop Med Public Health. 2010; 41:536-540.
 28. Preechaporn W, Jaroensutasinee M, Jaroensutasinee K. The Larval Ecology of *Aedes aegypti* and *Ae. albopictus* in Three Topographical Areas of Southern Thailand, 2006.
 29. Montgomery BL, Ritchie SA. Roof gutters: a key container for *Aedes aegypti* and *Ochlerotatus notoscriptus* (Diptera: Culicidae) in Australia. Am J Trop Med Hyg. 2002; 67(3):244-6.
 30. Thete KD, Shinde LV. Survey of container breeding mosquito larvae in Jalna City (M.S.), India, Biological Forum. 2013; 5(1):124-128.
 31. Dieng H, Saifur RG, Ahmad AH, Salmah MR, Aziz AT, Satho T, *et al.* Unusual developing sites of dengue vectors and potential epidemiological implications. Asian Pac J Trop Biomed. 2012; 2:228-232.
 32. Diagne CT, Faye O, Guerbois M, Knight R, Diallo D, Faye O, *et al.* Vector competence of *Aedes aegypti* and *Aedes vittatus* (Diptera: Culicidae) from Senegal and Cape Verde archipelago for West African lineages of chikungunya virus. Am J Trop Med Hyg. 2014; 91:635-641.