

A Survey of Mosquito Larval Habitats and Species Distribution in Rivers State University of Science and Technology Nkpolu Porthacourt, Nigeria

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Abstract: A survey was carried out to determine the various mosquito breeding habitats within the campus of the Rivers State University of Science and Technology and ascertain information on the public health importance of the mosquito species found in the area. Larval survey was carried out in four breeding habitats located within the staff residential quarters and security/Acada village of the university. The breeding habitats included stagnant pool of water, gutter/drains, discarded containers and plantain leaf axil. Dipping and Aspiration methods were used to sample mosquito larvae in the various habitats. The survey was carried out during the wet season from April-June, 2011. The larvae were sorted, counted and reared in the laboratory under ambient environmental conditions. The emerged adults were identified morphologically to species level using standard keys. Three genera (*Anopheles*, *Aedes* and *Culex*) of mosquitoes were got from the breeding habitats. Mosquitoes species identified included two species of *Anopheles*, *An gambiae* s.l and *An nilli*, three species of *Culex*, *Cx. quinquefasciatus*, *Cx poicilipes* and *Cx tigripes* and one species of *Aedes*, *Aedes aegypti*. *Aedes aegypti* was the most abundant (40.4%) and well distributed in all the breeding sites, this was followed by *An gambiae* s.l (31.84%) and *C. quinquefasciatus* (18.37%). *An nilli*, *C poicilipes* and *C tigripes* had percentage abundance of 5.71%, 2.86% and 0.19% respectively. Most of these species have been implicated in public health diseases. This suggests that the residents within the university are at risk of mosquitoes-borne diseases. The study recommends strict enforcement of proper waste management and health enlightenment campaigns within the university community which will reduce the breeding of these mosquitoes and reduce the transmission risk.

Keywords: Mosquito; Larva; Breeding-sites; University; Port Harcourt

1. Introduction

Mosquito are of public health importance as they constitute serious biting nuisance and transmit most deadly and life-threatening diseases such as malaria, denque and yellow fever, and bancroftian filariaris [1, 2]. Nigeria is known for high prevalence of malaria and the disease remains one of the leading causes of childhood and maternal morbidity and mortality, low productivity and reduced school attendance [3].

Mosquitoes exploit almost all types of lentic aquatic habitats for breeding and larvae of mosquitoes have been found to thrive in aquatic bodies such as fresh or salt water marshes, mangrove swamps, rice fields, grassy ditches, the edges of streams and rivers and small, temporary rain pools [4]. Unplanned urban growth, inadequate waste disposal, irrigation and poor drainage usually alter ecosystem and thus promote prolific breeding of mosquitoes [5].

The existence and distribution of the mosquitoes in the environment affects the quality of life of individuals, who may be inflicted by consequent morbidities and mortalities arising from the diseases borne by these vectors [6]. Constant studies on Biology and larval ecology of mosquitoes have been observed as important tools in mosquito control [7] and such studies will help to determine the existing and disappearing mosquito species, relative population densities and the extent of their distribution, seasonal trends and disease infection rates [8].

Larval control (source reduction) is a successful way of reducing mosquito densities by decimating the number of larvae that will emerge into adults [9]. To address the

problem of mosquito-borne diseases through larval control interventions, Fillinger and others [10] suggested the need to consider all potential larval habitats for larval control interventions, while Gu and Novak [11] argued for the need to identify all potential larval habitats and then direct larval control efforts to the most productive habitats.

For any vector control measures to be successful, good knowledge of the breeding ecology of mosquitoes including, the types and preferences for larval habitats, spatial and temporal distribution of breeding sites, as well as, the physical, biological and chemical characteristics of the habitats are required [12].

The key objective of this study was therefore to identify the breeding habitats and species composition of mosquitoes at the Rivers state university of Science and Technology, Port Harcourt and determine their medical importance.

2. Materials and Method

Study Site

The study was carried out within the Rivers State University of Science and Technology Nkpolu Port Harcourt, Rivers State, Nigeria. The area is located within the tropical rain forest zone with two marked seasons, the dry and wet. There are about eight (8) months (April – November) of wet season and four (4) months (November – March) of dry season. The vegetation is that of the tropical rain forest with an average annual rainfall of about 1600mm and an atmospheric temperature of 30°C.

Larval collections

Mosquito larvae were collected randomly from two sites within the university. The sites were the staff residential quarters and security village of Rivers State University of Science and Technology. In each of these sites, mosquito larvae were sampled from four locations making a total of eight locations. The locations were geo-referenced using a hand held GPS (Table 1). The breeding sites sampled were stagnant water pool, axil of plantain/bannana, gutter/drains, and discarded containers. The relative humidity and temperature of the locations were all measured and recorded. The vector sampling was concentrated in areas around human dwellings.

Table 1: Geographical locations of larval breeding sites

Staff Quaters	Security Village
04 ^o 47.493N 006 ^o 58.55E	04 ^o 48.360N 006 ^o 59.018E
04 ^o 47.553N 006 ^o 58.623E	04 ^o 48.357N 006 ^o 58.993E
04 ^o 47.569N 006 ^o 58.580E	04 ^o 48.274N 006 ^o 59.123E
04 ^o 47.594N 006 ^o 58.556E	04 ^o 48.386N 006 ^o 58.970E

Methods of larval collections

Samples were collected monthly in the first three months of rainy season, April, May and June, 2011. Mosquito larvae were collected using dipper and pipettes as described by Adeleke and others [9]. The larvae in leaf axils were collected with pipette while those in other breeding sites were collected by using standard dipper (400ml) recommended by WHO, [13]. Ten dips were collected from each of the breeding sites. The samples collected from each breeding habitat were pooled and poured into a plastic container and transported to Malaria Entomology Research laboratory of the Rivers State University of Science and Technology, Port Harcourt. In the laboratory, the mosquitoes were reared, fed and monitored till adult under standard environmental condition. On emergence, the adults were collected using an aspirator into plastic containers, killed with knock-down insecticides and subsequently transferred into Ependoff tube containing silica gel for preservation.

Identification of Mosquitoes

Identification of the adult mosquitoes was carried out microscopically using morphological keys by Gillett [14]. The identification was based on gross external morphological features, appearance of the antennae, palps, thorax, abdominal segment, colour of hind legs and striations on the body, proboscis etc.

3. Results

A total of 245 mosquitoes (Table 2) comprising three genera (*Anopheles*, *Aedes* and *Culex*) were got. Of this number, 32.65% (N=80) were sampled from plantain leaf axil, 36.33% (N=89) from stagnant water pool, 22.45% (N=55) from discarded containers and 8.57% (N=21) from gutter.

Table 2: Total Mosquitoes Abundance and Diversity in RSUST from April-June, 2011

Mosquitoes Species	Breeding sites			
	Plantain leaf axil	Stagnant water pool	Discarded container	Gutter
<i>Anopheles gambiae s.l</i>	20	31	18	9
<i>An. nile</i>	5	6	0	3
<i>C. quinquefasciatus</i>	13	16	11	5
<i>C. poicilipes</i>	7	0	0	0
<i>C. tigripes</i>	1	1	0	0
<i>Ae. aegypti</i>	34	35	26	4
Total	80	89	55	21
Percentage total	32.65%	36.33%	22.45%	8.5%

Mosquitoes species identified include two species of *Anopheles*, *An gambiae s.l* and *An nilli*, three species of *Culex*, *Cx. quinquefasciatus*, *Cx poicilipes* and *Cx tigripes* and one species of *Aedes*, *Aedes aegypti*. Analysis of variance (ANOVA) of the species abundance and distribution in the various habitats indicated that only *Aedes aegypti* and *Cx poicilipes* showed significant variations (P<0.05) while the rest did not vary significantly across the habitats.

The distribution of the genera (Fig 1) shows that *Aedes* are the most widely distributed (40.24%) in the area followed by *Anopheles* (37.8%) and *Culex* (21.95%).

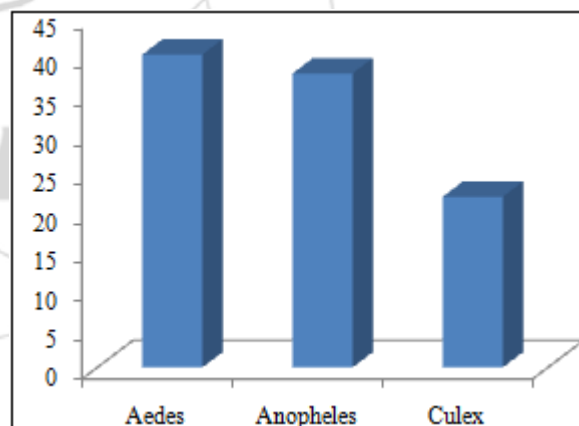


Figure 1: Distribution of mosquito genera in the various breeding habitat in RSUST

The distribution of mosquito species in the breeding sites indicate that more species were found in the plantain leaf axils (6), followed by stagnant water pool (5) and gutter/drains (4). The list population was obtained in the discarded containers. Among the mosquito larval species identified, *Aedes aegypti* was the most abundant (44%) and well distributed in all the breeding sites, this was followed by *An gambiae s.l* (31.84%) and *Cx. quinquefasciatus* (18.37%). *An nilli*, *Cx. poicilipes* and *Cx. tigripes* had percentage abundance of 5.71%, 2.86% and 0.19% respectively (Fig 2).

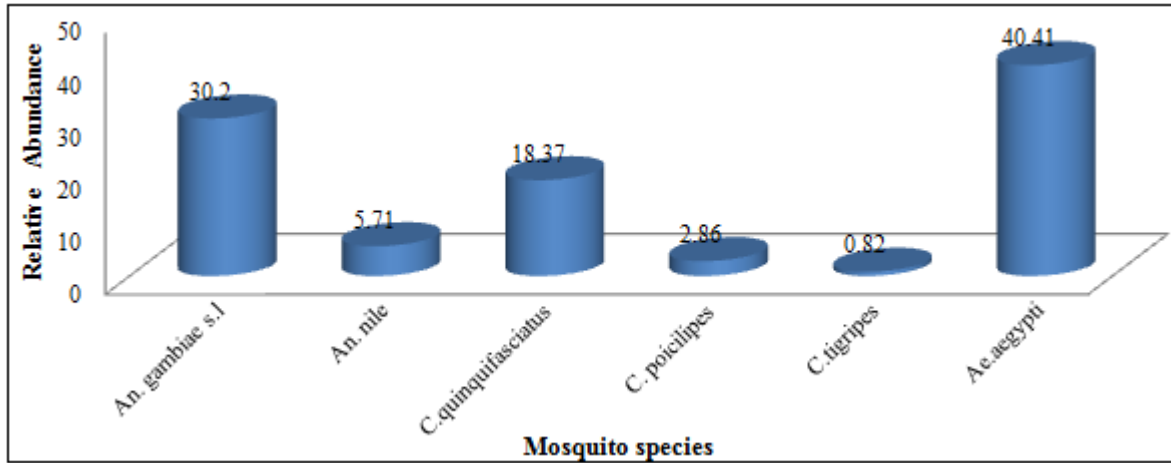


Figure 2: Mosquitoes species distribution in RSUST from April-June, 2011

Result from the stagnant pool of water (Fig.3) revealed that *Aedes aegypti* (39.32%) was the most occurring species in the habitat. This was followed by *An. gambiae s.l* (34.83%) and *Cx. quinquefasciatus* (18.0%). *An. nilli* and *Cx. tigripes* population was quite low.

Ae aegypti also dominated (39%) the stagnant pool of water in the study area (Fig.4), this was closely followed by *An gambiae s.l* (35%). *Cx. quinquefasciatus* constituted 18% of the mosquitoes sampled. *An nilli* and *Cx. tigripes* were sparsely distributed constituting about 7% and 1% of the population.

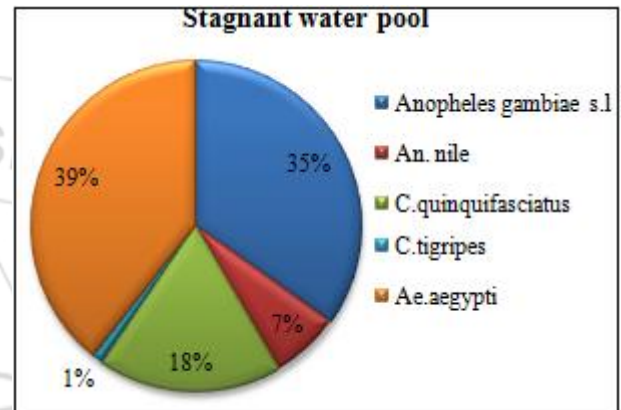


Figure 4: Mosquitoes species distribution in stagnant water in RSUST from April-June, 2011

Only three species of mosquitoes (*An gambiae*, *Cx quinquefasciatus* and *Ae aegypti*) were sampled in discarded containers with *Ae. Aegypti* dominating. *Cx. quinquefasciatus* was the least abundant (Fig.5).

Percentage distribution of the mosquito species found in drainage (Fig.6) revealed that *An. gambiae s.l* was the most dominant (43%). Other species present were *Cx. quinquefasciatus* (24%), *Ae aegypti* (14%) and *An. nilli* (14%) *Cx poicilipes* was only found in plantain leaf axil while a relatively low population of *Cx. tigripes* was found in plantain leaf axil and stagnant water. *An nilli* was found in all breeding sites except discarded containers.

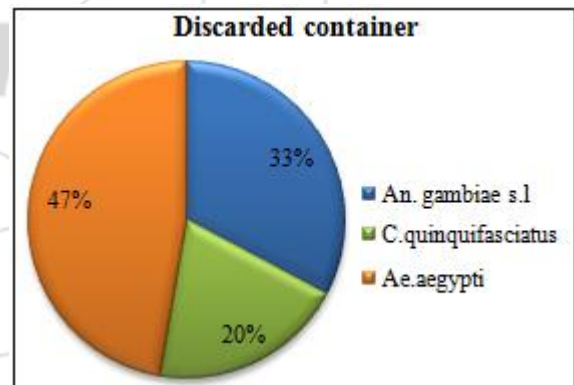


Figure 5: Mosquitoes species distribution in discarded container in RSUST from April-June, 2011

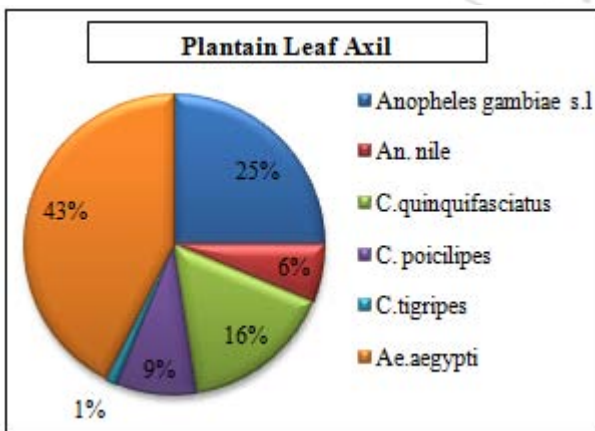


Figure 3: Mosquitoes species distribution in Plantain leaf axil in RSUST from May-June, 2011

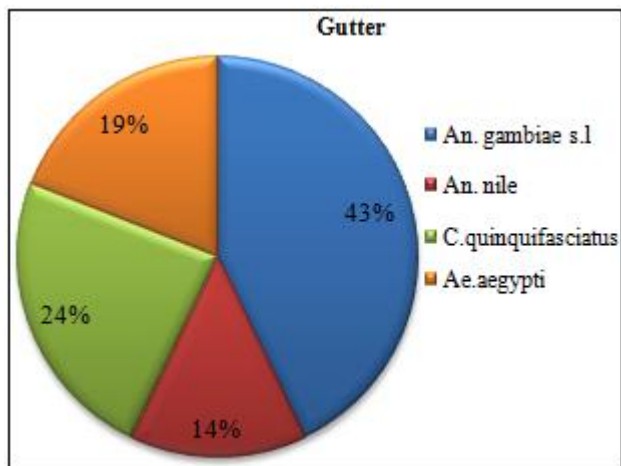


Figure 6: Mosquitoes species distribution in Gutter in RSUST from April-June, 2011

4. Discussions

The study reveals Stagnant water habitats to be the most favourable and successful habitat for the breeding of mosquitoes, followed slightly by plantain leaf axils habitats in the study area. This could be due to the fact that these habitats had with it, the necessary requirement for the growth of mosquito larvae, such as favourable temperature, availability of adequate food, water and other essential substance for their growth.

Discarded container was observed to be a favourable habitat for mosquito breeding in the study area compared to gutter habitats. This could have been influenced by the high rate of indiscriminate disposal of waste especially of tins, plastics in the security village which could retain enough water for the breeding of mosquito. The overall poor population of the mosquitoes in the gutter/drains could be as a result of the good drainage system in the university made of concrete which makes it unfavourable for mosquito breeding.

In general, the total abundance of mosquitoes in the habitats surveyed was poor. This could be attributed to severe rainfall that characterized the period. Rainfall washes out larvae from their breeding habitats leading to reduction in their density.

Larval survey revealed the co-breeding of *Aedes*, *Culex* and *Anopheles* in a variety of habitat. Generally, the mosquitoes showed preference for different breeding habitats. The disparity in the abundance and distribution of the mosquito species in the different locations could be due to different environmental conditions or microclimatic variations as well as availability of suitable preferred breeding sites.

The most dominant specie observed from the study was *Aedes aegypti*. The present result is in agreement with that reported elsewhere in Nigeria [9, 4, 15, 16, and 17]. The wide geographical distribution of *Ae. aegypti* is likely to be associated with variations in its biology [18, 19], enabling it to survive in a variety of different environmental conditions. This mosquito species show breeding preferences for domestic water containers [20]. Stored water in the container for long period and ambient relative humidity and temperature may favour the breeding of *Ae. aegypti* and

other aedine mosquitoes [21]. The types of the containers, water quality, and conditions of water containers are important for breeding [22]

Anopheles gambiae s.l. were the second dominant species in the studied habitat and well distributed. *Anopheles* mosquitoes in general breed in clean and oxygenated water. The abundance of such habitats on campus creates a favourable environment for the breeding of the mosquito species.

Culex was one of most frequently encountered genus in the present survey. Larvae belonging to this genus were present in quite high numbers throughout the survey period.

The presence of *Aedes*, *Culex* and *Anopheles* which are known vectors of urban yellow fever, lymphatic filariasis and malaria [23, 2] suggest that the residents within the study area are at risk of mosquito-borne diseases. Mosquito-borne disease to infect more than 700 million people around the world each year, and it results in as many as two million deaths annually.

Aedes aegypti is the most efficient vector for arboviruses because it is highly anthropophilic, frequently bites, and thrives in close proximity to humans [24]. It is the principal vector of dengue fever and dengue hemorrhagic fever in almost all countries [24, 25]. *Ae. aegypti* have also been implicated in large outbreaks of Zika virus disease. This is one mosquito species likely to give rise to an immediate public health problem if the authority fails to act appropriately within the study area.

Culex quinquefasciatus is of great concern to public health as diseases such as lymphatic filariasis have been traced to this particular specie in various part of the country. *Culex* spp. are known to be the most important vectors of West Nile virus (WNV) [26] of which infections are increasingly occurring in many parts of the world

Mosquitoes of the *An. gambiae* complex have been reported as important vectors of malaria and lymphatic filariasis (LF), which are major public health diseases in Nigeria. The abundance of the specie in this area could be associated to the favourable climatic conditions characteristics of the zone and abundance of breeding habitats.

Anopheles nili is a widespread efficient vector of *Plasmodium* parasites in the humid savannas and forested areas of sub-Saharan Africa [27-32]. *Anopheles nili* is usually responsible for a high nuisance to humans in villages along rivers, and abundance rapidly decreases within a few kilometers from the breeding sites [33]. It is considered a secondary malaria vector in Nigeria [34-37]. The anthropophilic habits of these vectors are a major contributing factor to their public health impact. It is possible that the *Anopheles nili* recorded in the study area may be of local importance in disease transmission.

The finding that mosquito larval species co-habit in the different habitats which are mostly man made suggested that larval control in the study area could be targeted to the breeding sites. In this instance, source reduction is the most

appropriate method of choice. Indoor residual spraying (IRS) is also recommended for severe epidemic situation.

5. Conclusions

This study has provided baseline information on the types of mosquito larval breeding habitats in the university community. It has also created the awareness about the vectors and the need for the university community to be protected. Furthermore this study calls for regular spraying of the breeding sites and the residential houses using WHO approved insecticides. The study recommends proper environmental management, health education, good drainage system and proper hygiene within the university community which will reduce the breeding of these mosquitoes. Regular surveillance is also needed to monitor the build up of larval populations in the area in order to reduce disease transmission.

References

- [1] Adeleke, M.A., Mafiana, C.F., Idowu., A.B., Sam-Wobo, S.O. and Idowu, O.A (2010): "Population dynamics of indoor sampled mosquitoes and their implication in disease transmission in Abeokuta, South-western Nigeria". *Journal of Vector Borne Disease*, 47:33-38.
- [2] World Health Organization (2012): Global strategy for dengue prevention and control 2012-2020. World Health Organisation, Geneva.
- [3] Aribodor, D.N., Nwaorgu, O.C., Eneanya, C.I., Aribodor O.D (2007): Malaria among Primigravid women attending antenatal clinic in Akwa, Anambra State, South-East Nigeria. *Nigeria Journal of Parasitology* 28(1):25-27.
- [4] Afolabi, O.J., Simon-Oke, I.A., and Osomo, B.O (2013): Distribution, abundance and diversity of mosquitoes in Akure, Ondo State, Nigeria. *Journal of Parasitology and Vector Biology* 5(10)132-136.
- [5] Adeleke, M. A., Adebimpe, W. O., Hassan, A. O., Oladejo, S. O., Olaoye, I. K., Olatunde, G. O. & Adewole, T. A (2013): Larval ecology of mosquito fauna in Osogbo, Southwestern Nigeria. *Asian – Pacific Journal of Tropical Biomedicine*, 3 (9): 673-677.
- [6] Sia Su, G; Bobadilla, J ;De Leon, K.C ; Sia Su, M.L and Rragio, E (2014): Larval Mosquito Diversity and Distribution in Rice Field Agro-Ecosystems in Sariaya, Quezon Province, Philippines, *Annual Research & Review in Biology* 4(18): 2884-2891.
- [7] Kim, H.C., S.T. Chong, P.V. Nunn and T.A. Klein (2010): Seasonal prevalence of mosquitoes collected from light traps in the Republic of Korea, 2007. *Entomol. Res.*, 40: 136-144.
- [8] Ekesiobi, A. O., Anene, C .C., Nwigwe, H. C., Emmy-Egbe, I.O., and Igbodika, M. C (2014): Seasonal Distribution and Abundance of Yellow Fever Mosquito Vector, *Aedes aegypti*, (Diptera: Culicidae) In Aguata L .G.A, Anambra State. *COOU Interdisciplinary Research Journal*, Vol. 1 No 1
- [9] Adeleke MA., Mafiana CF., Idowu AB., Adekunle MF., and Sam-Wobo SO (2008): Mosquito larval habitats and public health implication in Abeokuta, Ogun State, Nigeria. *Tanzan J Health Res.* 10(2):103–107.
- [10] Fillinger U., George S., Gerry FK., Bart GJK., and Norbert B (2004): The practical importance of permanent and semipermanent habitats for controlling aquatic stages of *Anopheles gambiae sensu lato* mosquitoes: operational observations from a rural town in western Kenya. *Trop Med Int Health* 9:1274–1289
- [11] Gu W, and Novak RJ (2005): Habitat-based modeling of impacts of mosquito larval interventions on entomological inoculation rates, incidence, and prevalence of malaria. *Am J Trop Med Hyg* 73: 546–552.
- [12] Olayemi IK., Omalu ICJ., Famotele, OI., Shegna SP., and Idris B (2010): Distribution of mosquito larvae in relation to physico-chemical characteristics of breeding habitats in Minna, North Central, Nigeria. *Rev. Infect.* 1(1):49-53. S
- [13] World Health Organization (2005): Guidelines for laboratory and field testing of mosquito larvicides. WHO/CDS/WHOPES/GCDPP/2005.13
- [14] Gillet, J.D (1972): Common African Mosquitoes and their Medical Importance. William Heinemann Medical Books Limited, London. Volume I, Pp. 106.
- [15] Aribodor, D. N., Aribodor, O. B., Eneanya C. I. and Onyido, A. E (2013): Survey of Open Larval Habitats of Mosquitoes in Four Communities in Awka South Local Government Area, Anambra State, Nigeria The Bioscientist: Vol. 1(2):132-139, September.
- [16] Mafiana, C.F., Anaeme, L. and Olatunde, G.O (1998): Breeding sites of Larval Mosquitoes in Abeokuta, Nigeria. *Nigeria Journal of Entomology* 15, 136-143.
- [17] Okorie, T.G (1978): "The breeding site preference of mosquito in Ibadan, Nigerian". *Nigeria Journal of Entomology*, 3:1-80. WHO, Dengue Guidelines for Diagnosis, Treatment, Prevention and Control, WHO, Geneva, Switzerland.
- [18] Glasser, C. M. and A. C. Gomes (2002): Climate and the superimposed distribution of *Aedes aegypti* and *Aedes albopictus* on infestation of São Paulo State, Brazil. *Revista de Saúde Pública* 36:166-172.
- [19] Beserra, E. B., F. P. Castro Jr., J.W. Santos., T. S. Santos and C. R. M. Fernandes (2006): Biologia e Exigências Térmicas de *Aedes aegypti* (L.) (Diptera: Culicidae) Provenientes de Quatro Regiões Bioclimáticas da Paraíba. *Neotropical Entomology* 35: 853-860.
- [20] World Health Organization (2002): Dengue Fever and Dengue Haemorrhagic Fever Prevention and Control. WHO, Geneva. [accessed 3 March 2008]
- [21] Dejene Getachew., Habte Tekie., Teshome Gebre-Michael., Meshesha Balkew., and Akalu Mesfin (2015): Breeding Sites of *Aedes aegypti*: Potential Dengue Vectors in Dire Dawa, East Ethiopia. *Interdisciplinary Perspectives on Infectious Diseases* Gubler, D. J. 2002. Epidemic dengue/ dengue hemorrhagic fever as a public health, social and economic problem in the 21st century. *Trends of Microbiology* 10: 100-103 Article ID 706276, 8 pages
- [22] Chen, H. L. Lee., S. P. Stella-Wong., K. W. Lau., and M. Sofian-Azirun (2009): "Container survey of

- mosquito breeding sites in a university campus in Kuala Lumpur, Malaysia,” *Dengue Bulletin*, vol. 33, no. 1, pp. 187–193.
- [23] Service, M.W (1993): Mosquitoes (Culicidae) in lane RP, Crosskey, RW, editors. *Medical insects and arachnids*. London: Chapman & Hall, p. 120-240.
- [24] World Health Organisation (2009): Dengue, guidelines for diagnosis, treatment and prevention: A joint publication of the WHO and the Special programmes for research and training in tropical diseases (TDR)
- [25] Were (2012) “The dengue situation in Africa,” *Paediatrics and International Child Health*, vol. 32, supplement s1, pp. 18–21.
- [26] Diaz-Badillo, A., Bolling, B.G., Perez-Ramirez, G et al (2011): The distribution of potential West Nile virus vectors, *Culex pipiens pipiens* and *Culex pipiens quinquefasciatus* (Diptera: Culicidae), in Mexico City. *Parasites & Vectors* 14:70
- [27] Antonio-Nkondjio C., Keraf Hinzoumbe C., Simard F., Awono-Ambene P., Tchuinkam T., and Fontenille D (2006): Complexity of the malaria vectorial system in Cameroon: Contribution of secondary vectors to malaria transmission. *Journal of Medical Entomology* 43:1215-1221.10.1603/0022-2585(2006)43[1215:COTMVS]2.0.CO;2.
- [28] Carnevale P., Le Goff G., Toto JC., and Robert V (1992): Anopheles nili as the main vector of human malaria in villages of southern Cameroon. *Med Vet Entomol.*, 6: 135-138. 10.1111/j.1365-2915.tb00590.
- [29] Dia I., Diop T., Rakotoarivony I., Kengne P., and Fontenille D (2003) Bionomics of *Anopheles gambiae* Giles, *An. arabiensis* Patton *An. funestus* Giles and *An. nili* (Theobald) (Diptera: Culicidae) and Transmission of *Plasmodium falciparum* in a Sudano-Guinean Zone (Ngari, Senegal). *J Med Entomol.*, 40: 279-283. 10.1603/0022-2585-40.3.279.
- [30] Adja AM., N'goran KE., Kengne P., Koudou GB., Toure M., Koffi AA., Tia E., Fontenille D., and Chandre F (2006): Transmission vectorielle du paludisme en savane arborée à Gansé en Côte d'Ivoire. *Med Trop.*, 66: 449-455.
- [31] Moffett A., Shackelford N., and Sarkar S (2007): Malaria in Africa: vector species' niche models and relative risk maps. *PLoS ONE*. 2: e824-10.1371/journal.pone.0000824.
- [32] Ayala D., Carlo Costantini., Ose K., Kamdem GC., Antonio-Nkondjio C., Agbor JP., Awono-Ambene P., and Fontenille D, Simard F (2009): Habitat suitability and ecological niche profile of major malaria vectors in Cameroon. *Malar J.*, 8: 307-10.1186/1475-2875-8-307.
- [33] Le Goff G., Carnevale P., and Robert V (1997): Low dispersion of anopheline malaria vectors in the African equatorial forest. *Parasite*. 2:187 - 189.
- [34] Boreham P. E., Lenahan J. K., Boulzaquet R., Storey J., Ashkar T. S., Nambiar R., and Matsu Shima T (1979): Studies on multiple feeding by *Anopheles gambiae* s.l. in a Sudan Savannah area of northern Nigeria. *Trans R Soc Trop Med Hyg*, 73, 418– 423.
- [35] Molineaux, L and Gramiccia, G. (1980): The Garki project. Research on the epidemiology and control of malaria in the Sudan Savannah of West Africa. Geneva, Switzerland: World Health Organization; p. 311.
- [36] Gillies MT., Coetzee M (1987): Supplement to the Anophelinae of Africa South of the Sahara (Afrotropical region). *Publ S Afr Inst Med Res*, 55:143.
- [37] World Health Organization (1992): Entomological Field Techniques for Malaria Control: Part 1 Learner's Guide. World Health Organization, Geneva.
- [38] Gillies, M.T. and De Meillon, D. (1968): The Anophelinae of Africa South of the Sahara. *Publication of the South African Institute of Medical Research* 54:343.