International Journal of Mosquito Research

ISSN: **2348-5906**
CODEN: **IJMRK2**
IJMR 2020; 7(4): 01-05
www.dipterajournal.com

Received: 01-05-2019
Accepted: 03-06-2019

Lawal Nura
(1). Department of Biochemistry & Molecular Biology, Federal University Dutsinma Katsina State Nigeria
(2). Department of Biochemistry, Bayero University Kano, Nigeria

Ali Siddiq Idoko
Department of Biochemistry & Molecular Biology, Federal University Dutsinma Katsina State Nigeria

Abdullahi Hadiza
TETFUND, Abuja, Nigeria

Imam Abdullahi Abdulqadir
Professor, Department of Biochemistry, Bayero University Kano, Nigeria

Corresponding Author:
Imam Abdullahi Abdulqadir
Professor, Department of Biochemistry, Bayero University Kano, Nigeria

Insecticide susceptibility bioassay in *Culex quinquefasciatus* vector of lymphatic filariasis from Sahel Savannah region of Northwest Nigeria

Lawal Nura, Ali Siddiq Idoko, Abdullahi Hadiza and Imam Abdullahi Abdulqadir

Abstract
Lymphatic filariasis (LF) is a disease of significant public health importance, transmitted by both *Anopheles* and *Culex* mosquitoes. The aim of this study is to identify the *Culex* mosquito *spp* morphologically and by using PCR and to assess the insecticides susceptibility status of *Culex* mosquitoes. A total of 350 mosquitoes were collected by indoor collection methods using mechanical aspirator and F_1 of these collected *Culex* mosquitoes were used to performed WHO bioassay. Mosquitoes were morphologically and molecularly identified at 274bps as *Culex quenquifasciatus* from Batagarawa town and Gajerar Giwa village. *Culex quenquifasciatus* from Batagarawa town are highly resistance to permethrin and DDT recorded 15.39% and 18.12% but, highly susceptible to deltamethrin, bendiocarb, propoxur and malathion recorded 56.68%, 96.4%, 80% and 96.4% knockdown after one hour exposure with mortality rate recorded 21.7%, 32.4%, 96.9%, 96.9%, 80.0% and 100% after 24 hours post exposure respectively. However, *Culex quenquifasciatus* from Gajerar Giwa village are highly resistance to permethrin and DDT recorded 15.39% and 18.12% but, highly susceptible to deltamethrin, bendiocarb, propoxur and malathion recorded 90%, 56.12%, 77.7% and 89.10% knockdown after one hour exposure, and low mortality was observed with permethrin and DDT recorded 14.9% and 32.5% and then high mortality with deltamethrin, bendiocarb, propoxur and malathion recorded 96.5%, 56.12%, 77.7% and 89.10% after 24 hours post exposure respectively. Permethrin and DDT are no longer effective in vector control programme but, deltamethrin, bendiocarb, propoxur and malathion are still effective in vector control strategy.

Keywords: *Culex quenquifasciatus,* deltamethrin, bendiocarb, propoxur

Introduction
Lymphatic filariasis (LF) is a major public health problem caused by filarial parasites; *Wuchereria bancrofti, Brugia malayi or Brugia timori* and is presently endemic in 72 countries [1]. Mosquito species belonging to the *Anopheles, Culex, Aedes, Mansonia, Coquillettidia* and *Ochlerotatus genera* are carriers of the LF parasites. *Anopheles* mosquitoes (vectors of malaria) are the main vectors of LF in West Africa [2-3]. These mosquito species are distributed across tropical and subtropical regions in Africa where they also act as vectors of the *Wuchereria bancrofti* parasites that cause Lymphatic Filariasis (LF). *Culex* spp are also responsible for LF transmission, especially the abundant populations of *Culex quinquefasciatus* that often proliferate in dirty environments of human settlements [4].
Effort has been made to eliminate both malaria and LF, by combining curative drugs with indoor vector control measures such as long-lasting insecticidal nets (LLINs) and indoor residual spraying (IRS), which successfully reduced the health burden of these diseases[5-7] elimination of either is probably impossible without additional interventions to prevent outdoor transmission of the disease [8-10].
Volatile insecticides could complement with LLINs and IRS because they have a different mode of action which lends itself to outdoor application. Volatile active ingredients with repellent or toxic modes of action may be delivered in vapour phase so they protect spaces by diffusion through the air, rather than relying on mosquito contact with structural surfaces in the way as conventional, solid-phase contact insecticides [11-12].

It has been reported that insecticide resistance among the various vectors of LF exists on the African continent. The pyrethroid resistance mechanism of *kdr* mutation had been found distributed in the M and S forms of *An. gambiae s.s*, [13-15]. DDT and pyrethroid resistance have been widely observed in Africa, in *An. gambiae s.s* and *An. arabiensis*, with multiple-resistance mechanisms observed in West Africa [16]. These resistance mechanisms may inadvertently influence the density dependent processes and the vector competence of various *Anopheles* species. Studies have suggested that highly elevated esterases involved in insecticide resistance may inhibit development of mf in *Culex* [17], and similar effects could occur in insecticide resistant *Anopheles* [18-19]. This study was aimed to determine the distribution and insecticides susceptibility status of *Culex* mosquito from Northwest Nigeria.

Methods and Materials

Study Site and Mosquito Collection

Blood fed female *Culex* mosquitoes resting indoor were collected using mechanical aspirator from randomly selected houses, in the early morning hours (5:00 am–6:00 am) in two separate localities within the Sahel savannah region of Northwest Nigeria

(Figure 1): i) Batagarawa town, Batagarawa Local Government in the Sahel Savannah of Katsina State (12°54′17″N, 7°37′11″E), is a semi- urban area characterized by large number of *Culex* mosquitoes breeding sites; ii) Sahel Savannah of Gajerar Giwa village (12^0 95′21′N, 7^0 75′19′E) in Rimi Local Government of Katsina State, where rice and vegetables irrigation are practiced using water from Ajiwa dam, characterized by fewer of *Culex* mosquitoes breeding sites.

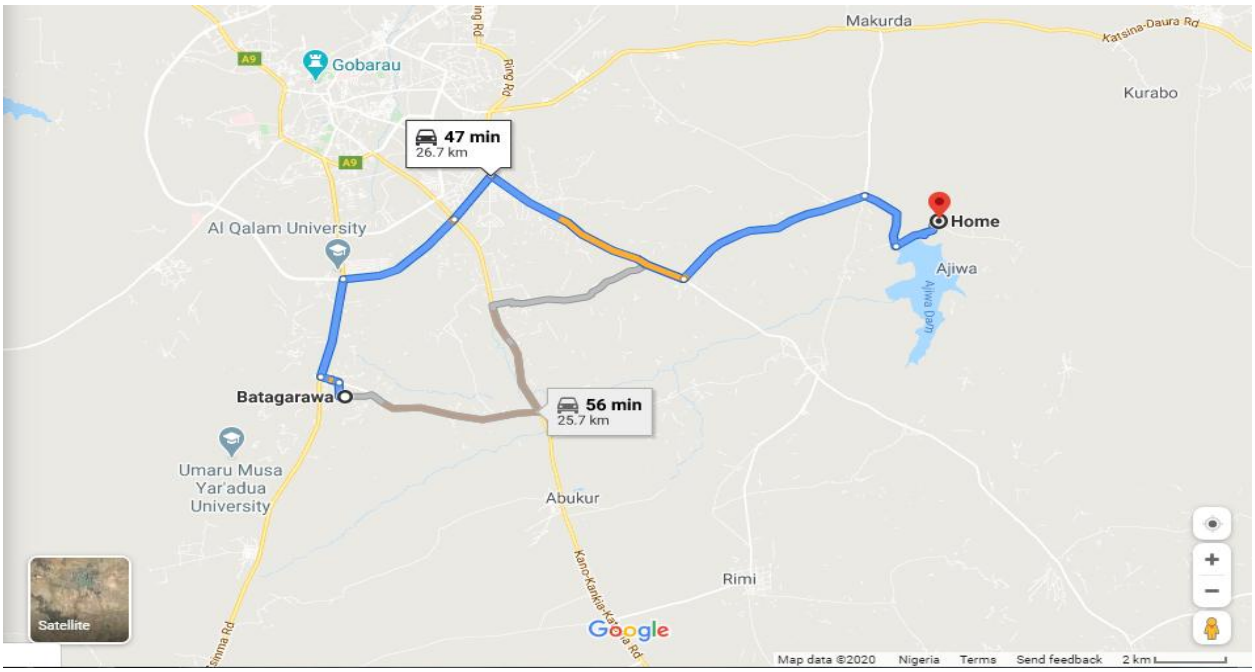

Fig 1: Adopted from google map

Mosquito Collection and Rearing

Collections were conducted at Batagarawa town for 21 days, from the month of April in 2017 to the month of April in 2018. For Gajerar Giwa villages, indoor collections were done for 17 days from the month of August in 2017 to the month of February in 2018.

The blood fed female *Culex* mosquitoes were maintained on 10% sugar at 25 °C ± 2 and 70–75% relative humidity for 6 days, until fully gravid. They were then transferred into 1.5 ml tubes individually and forced to lay eggs, as described previously [20].

All F_0 parents were identified as belonging to *Culex* mosquitoes using morphological keys. Egg batches were transferred into paper cups for hatching in insectary at Bayero University Kano, Nigeria. Hatched eggs were pooled into larvae bowls and supplemented with Tetramin^{TM} baby fish food. 2- to 4-days old F_1 females that emerged were randomly mixed in cages and used for bioassay experiments.

PCR-based Species Identification

Following morphological identification, Genomic DNA was extracted from mosquitoes obtained from Batagarawa town and Gajerar Giwa village which survived exposure to permethrin, using the LIVAK method [21]. Molecular species identification was carried out with the reaction volume of 20µl. The cycling conditions used were 95 °C for 5 min followed by 40 cycles of denaturation at 94 °C for 30 sec, annealing at 54 °C for 30 sec and extention at 72 °C for 10 min then a final extention at 72 °C for 10 min. Electrophoresis using 1.5% agarose gel stained in ethidium bromide was run for 30 min after loading 3 µl PCR product.

Insecticide Susceptibility Bioassays

Insecticide susceptibility assays were carried out using 2–4 day-old F1 adult's mosquitoes following the WHO protocol [22]. Approximately 20–25 mosquitoes per tube with 3–4 replicates were exposed to insecticide-impregnated papers for 1 h or control paper and then transferred to a clean holding tube supplied with 10% sugar and mortality rate was determined 24 hours post-exposure. The following insecticides were tested: the pyrethroids; permethrin (0.75%), and deltamethrin (0.05%); the carbamate; bendiocarb (0.01%); the organophosphate; malathion (5%) and the organochlorines; DDT (4%).

Results

Molecular Identification of Mosquito Species:

Out of the large number of *Culex spp* that were morphologically identified, 50 *Culex* mosquitoes were randomly selected for molecular specie identification. The results showed 47 (92.5%) in figures 2 and 3 were predominantly *Culex quenquifasciatus* from Batagarawa town and Gajerar Giwa village.

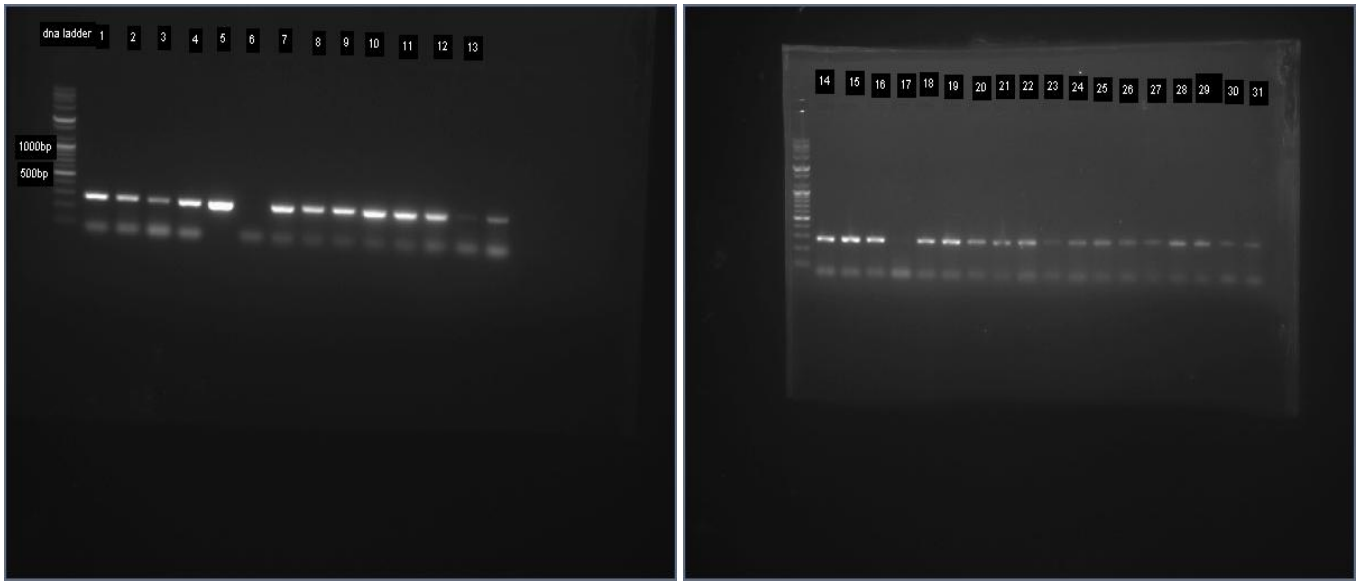

Fig 2: Gel electrophoregram for the identification of members *Culex spp* complex. Lane M = 100bp molecular weight marker, Lane 1-31 at 274bps= *Culex quenquifasciatus*

Insecticide impregnated paper

Test paper (12-15 cm^2) of insecticides at operational field concentrations was prepared according to the WHO protocol [23-24].

Insecticide Susceptibility Bioassays

Insecticides bioassay performed using the F_1 adult's female *Culex quenquifasciatus* from Batagarawa town, were more resistance to permethrin and DDT recorded 15.39% and 18.12%, but, highly susceptible to deltamethrin, bendiocarb, propoxur and malathion recorded 56.68%, 96.4%, 80% and 96.4% knockdown after one hour exposure respectively (Figure 3) and percentage mortality were 21.7%, 32.4%, 96.9%, 96.9%, 80.0% and 100% after 24 hours respectively (Figure 3). No mortality was recorded in control tubes. F_1 *Culex quenquifasciatus* from G/Giwa are highly resistance to permethrin and DDT recorded 15.39% and 18.12% but, high susceptibility was observed with deltamethrin, bendiocarb, propoxur and malathion recorded 90%, 56.12%, 77.7% and 89.10% knockdown after one hour exposure respectively (Figure 4). And low mortality was observed with permethrin and DDT recorded 14.9% and 32.5% and then high mortality with deltamethrin, bendiocarb, propoxur and Malathion recorded 96.5%, 56.12%, 77.7% and 89.10% after 24 hours post exposure respectively (Figure 4). No mortality was recorded in control tubes.

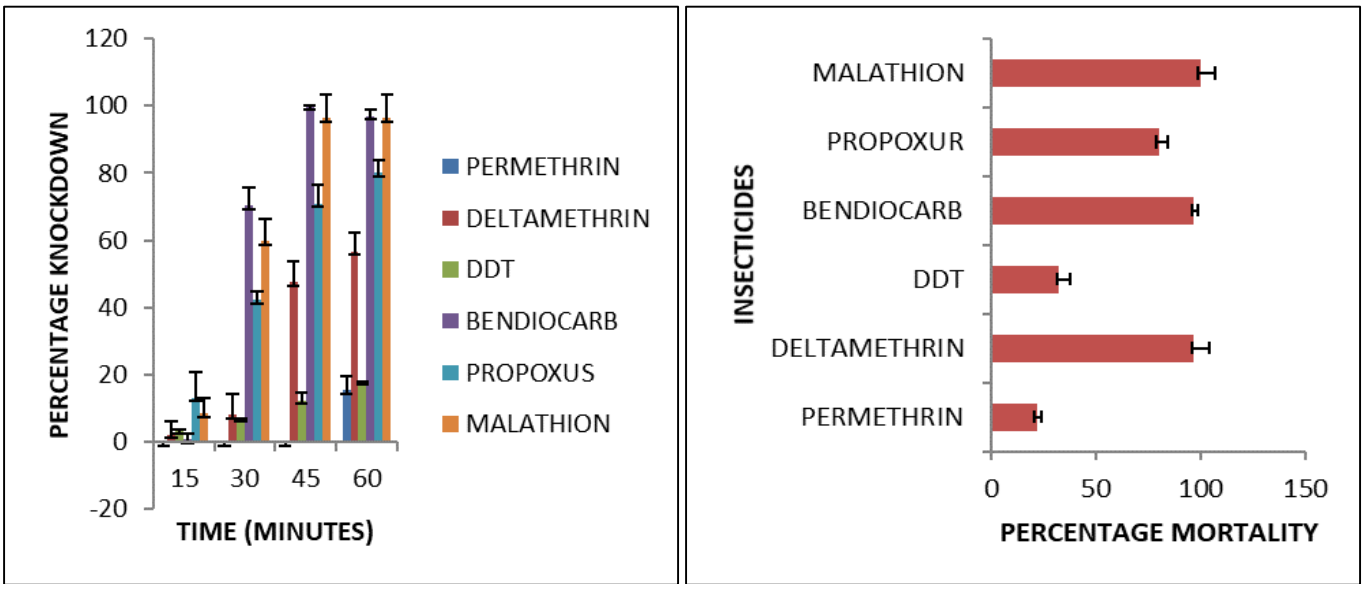

Fig 3: Knockdown profile and Insecticide susceptibility/resistance status of *Culex quenquifasciatus* from Batagarawa town. Error bars represent variability in the data.

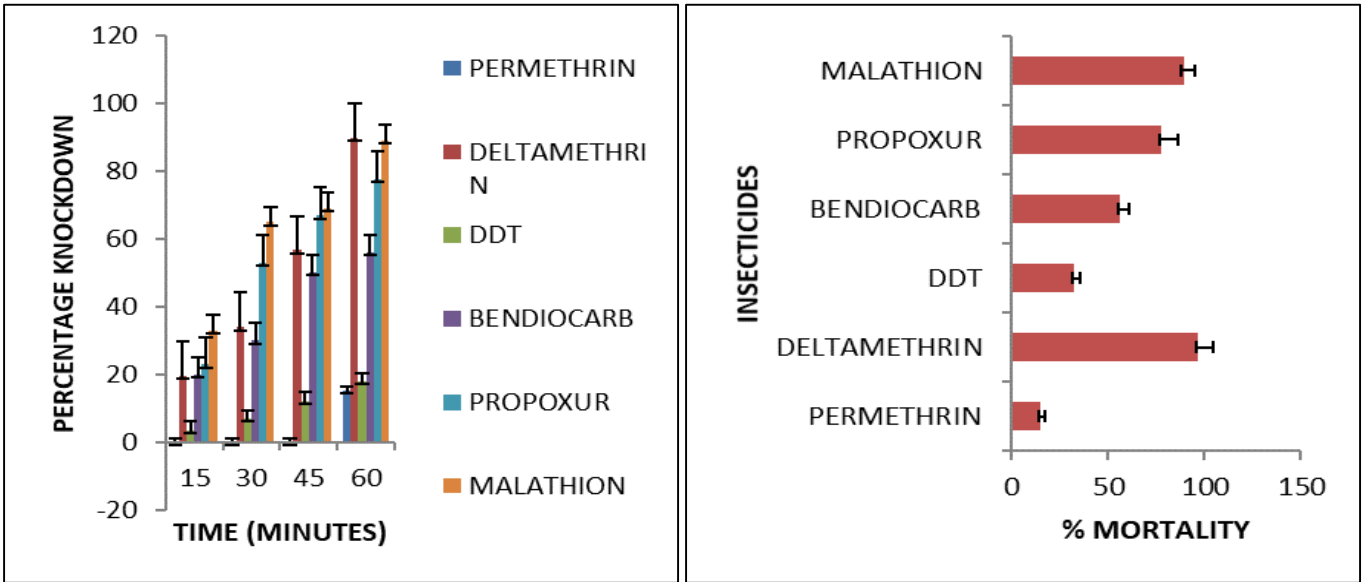

Fig 4: Knockdown profile and Insecticide susceptibility/resistance status of *Culex quenquifasciatus* from G/Giwa village. Error bars represent variability in the data.

Discussion

The susceptibility test status of mosquitoes is one of the major factors affecting the success of vector control strategy. For many years, synthetic pyrethroids and organochlorines have demonstrated great promise for mosquito vector control because of their low toxicity to humans and high potency at low doses quickly immobilizing and killing mosquitoes [25-26]; however, overtime, resistance to these synthetic compounds has been recorded in several species of arthropods, including *Culex quinquefasciatus*, *Anopheles Gambiae s.l* and *Anopheles Funestus s.l* complex.

In this study, it was clearly seen that most F_1 collected *Culex quinquefasciatus* from Batagarawa town and Gajerar Giwa village demonstrated comparatively high levels of resistance to permethrin and DDT. This could be due to cross-resistance between insecticides. It is found that permethrin and DDT share the same target site which is dependent sodium volte-gate channels of the nerve sheath [27]. Moreover, the breeding sites of these mosquitoes may be contaminated with agricultural allied chemicals and insecticides that have been used for pest control. Similar observation of permethrin and DDT cross resistance in *Culex quinquefasciatus* from Thailand was reported by Sunaiyana *et al.* [28] The existence of DDT and permethrin resistances in Batagarawa town and Gajerar Giwa village surveyed is a confirmation of the spatiotemporal spread of DDT and permethrin resistances in Sahel Savannah region of Northwest Nigeria. In the case of susceptibility to deltamethrin, previous studies have shown that same mosquito populations can be resistant and also susceptible to different insecticides from the same family. This may be attributed to cytochrome P450 (CYP6P4) up-regulated in this population and to be preferential to metabolized permethrin but not deltamethrin. Full susceptibility observed with deltamethrin indicates that *kdr* alone might not be sufficient to confer resistance to pyrethroid; it is likely that metabolic resistance is also involved contrary to what was reported by Okorie *et al.*, [29] from Ibadan, Nigeria that WHO bioassay results indicated high resistance to pyrethroids in both Type I (permethrin) and Type II (deltamethrin) pyrethroids.

Full susceptibility to bendiocarb, propoxur and malathion which were observed in F_1 adult mosquitoes caught from the two study locations may be attributed to over production of acetylcholinesterase enzyme in the nerve synapses and it may also be due to down regulation of detoxification enzymes. And also similar scenario was reported in Thailand that *Culex quinquefasciatus* mosquitoes were highly susceptible to malathion and propoxur, suggesting that these compounds may still be effective in controlling *Culex quinquefasciatus* [30].

Conclusion

With this spectrum of resistance to permethrin and DDT in *Culex quinquefasciatus*, it's inevitably moving towards a lack of effectiveness of permethrin and DDT used in the fight of LF vector. However, deltamethrin, propoxur, bendiocarb and malathion may still be effective in controlling *Culex quinquefasciatus* vector of LF. Integrated vector control strategy should be considered and resistance surveillance in mosquito needs to be conducted regularly.

References

1. World Health Organization. Lymphatic Filariasis. Fact Sheet No. 2012, 102.
2. Subramanian S, Krishnamoorthy K, Ramaiah KD, Habbema JDF, Das PK, Plaisier AP. The relationship between microfilarial load in the human host and uptake and development of *Wuchereria bancrofti* microfilariae by *Culex quinquefasciatus*; a study under natural conditions; *Parasitology*. 1998; 116:243-255.
3. Appawu MA, Dadzie SK, Wilmot-Baffoe A, Wilson MD. Lymphatic Filariasis in Ghana; Entomological investigation of transmission dynamics and intensity in communities served by irrigation systems in the Upper East Region of Ghana. Tropical Medicine International Health. 2001; 6:511-516.
4. Govella NJ, Ogoma SB, Paliga J, Chaki PP, Killeen G. Impregnating hessian strips with the volatile pyrethroid transfluthrin prevents outdoor exposure to vectors of malaria and lymphatic filariasis in urban Dares Salaam, Tanzania, Parasites & Vectors. 2015; 8:322.
5. Simonsen PE, Malecela MN, Michael E, Mackenzie CD.

Lymphatic Filariasis research and control in Eastern and Southern Africa. DBL-Centre for Health Research and Development. 2008.
6. World Health Organization. Global Programme to Eliminate Lymphatic Filariasis. In; Lymphatic Filariasis; Practical Entomology. 2013a; Geneva.
7. World Health Organization. World Malaria Report. 2014; *In*.
8. Bockarie MJ, Pedersen EM, White GB, Michael E. Role of vector control in the global program to eradicate lymphatic filariasis. Annual Review Entomology. 2009; 54:469-87.
9. Simonsen PE, Pedersen EM, Rwegoshora RT, Malecela MN, Derua YA, Magesa SM. Lymphatic filariasis control in Tanzania: effect of repeated mass drug administration with Ivermectin and Albendazole on infection and transmission. PLoS Neglected Tropical Diseases. 2010; 4:696.
10. Govella NJ, Ferguson H. Why use of interventions targeting outdoor biting mosquitoes will be necessary to achieve malaria elimination. Front Physiology. 2012; 3:1-5.
11. Pates HV, Line JD, Keto AJ, Miller JE. Personal protection against mosquitoes in Dares Salaam, Tanzania, by using a kerosene oil lamp to vaporize transfluthrin. Medical Veterinary Entomology. 2002; 16:277-84.
12. Killeen GF, Chitnis N, Moore SJ, Okumu FO. Target product profile choices for intra-domiciliary malaria vector pesticide products: repel or kill. Malar J. 2011; 10:207.
13. Weill M, Chandre F, Brengues C, Manguin S, Akogbeto M, Pasteur N *et al*. The *kdr* mutation occurs in the Mopti form of *Anopheles gambiae s.s*. through introgression. Insect Molecular Biology. 2000; 9(5):451-455.
14. Diabate A, Baldet T, Chandre C, Dabire KR, Kengne P, Guiguemde TR *et al*. *Kdr* mutation, a genetic marker to assess events of introgression between the molecular M and S forms of *Anopheles gambiae* (Diptera: Culicidae) in the tropical savannah area of West Africa. *Journal Medical Entomology*. 2003; 40(2):195-198.
15. Awolola TS, Oduala AO, Oyewole IO, Obensa JB, Amajoh CN, Koekemoer LL *et al*. Dynamics of knockdown pyrethroid insecticide resistance alleles in a field population of *Anopheles gambiae s.s*. in south-western Nigeria. Journal Vector Borne Diseases. 2007; 44(3):181-188.
16. Yewhalaw D, Wassie F, Steurbaut W, Spanoghe P, Van Bortel W, Danis L *et al*. Multiple insecticide resistance; an impediment to insecticide-based malaria vector control program. PLoS ONE. 2011; 6:1-10.137/journal.Pone.0016066.
17. McCarroll L, Paton MG, Karunaratne SHPP, Jayasuryia HTR, Kalpage KSP, Hemingway J. Insecticides and mosquito-borne disease. Nature. 2000; 407(6807):961-962.
18. Hemingway J, Hawkes N, Mc Carroll L, Ranson H. "The molecular basis of insecticides resistance in mosquitoes". Insect Biochemistry & Molecular Biology. 2004; 34:653-65.
19. Kelly-Hope LA, Diggle PJ, Rowlingson BS, Gyapong JO, Kyelem D, Coleman M *et al*. Negative spatial association between lymphatic filariasis and malaria in West Africa. Tropical Medicine International Health. 2006; 11(2):129-135.
20. Morgan JC, Irving H, Okedi LM, Steven A, Wondji CS. Pyrethroid resistance in an Anopheles funestus population from Uganda. PLoS One. 2010; 5:e11872, https://doi.org/10.1371/journal.pone.0011872.
21. Livak KJ. Organization and mapping of a sequence on the Drosophila melanogaster X and Y chromosomes that is transcribed during spermatogenesis. Genetics. 1984; 107(4):611-634.
22. World Health Organization. Test procedures for insecticide resistance monitoring in malaria vector mosquitoes. Geneva. 2013b; World Health Organization.
23. World Health Organization. WHO/CDS/WHOPES/98.10. 1998; Geneva.
24. World Health Organization. WHO/CDS/NTD/WHOPES/GCDPP/3. 2006; Geneva.
25. Prasittisuk M. Efficacy of three insecticides against Anopheles dirus and Anopheles minimus, the major malaria vectors, in Kanchanaburi Province Thailand. "Thailand Mahidol University". 1994; (Ph. D. Thesis).
26. Grieco JP, Archee NL, Chareonviriyaphap T, Suwankerd W, Chauha K, DR. Roberts DR. http://www.plosone.org. 2007.
27. Wondji SC. Two Duplicated P450 Genes are Associated with Pyrethroid Resistance in *Anopheles funestus*. a major malaria vector. Genome Res. 2009; 19(3):452-9.
28. Sunaiyana S, Pungasem P, Kasin S. "Detection of insecticides resistance status in *Culex quinquefasciatus* and *Aedes aegypti* to four major groups of insecticides". Tropical Biomedicine. 2006; 23(1):97-101.
29. Okorie PN, Ademowo OG, Irving H, Kelly-Hope LA, Wondji CS. Insecticide susceptibility of *Anopheles coluzzii* and *Anopheles gambiae* mosquitoes in Ibadan, Southwest Nigeria. Medical and Veterinary Entomology. 2015; 29:44-50
30. Kanutcharee T, Sunaiyana S, Theeraphap C. Insecticide resistance of *Aedes aegypti* and *Culex quinquefasciatus* in Thailand. J Pestic. Sci. 2008; 33(4):351-356.