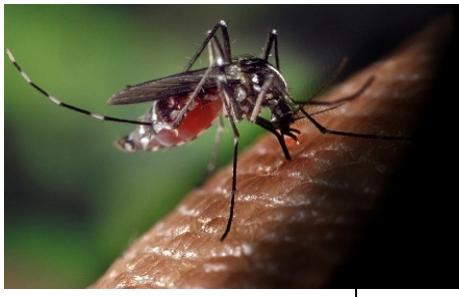

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Insecticides Susceptibility status of *Ae. aegypti*, *An. gambiae complex*, and *Culex* sp in West Darfur, Sudan

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Abstract
An experimental study was carried out in El geneina town, west Darfur state, Sudan, during the period of October - December 2018. This study aimed to determine the susceptibility status of *Ae. aegypti*, *An. gambiae complex* and *Culex* sp against four insecticide groups (Carbamates, pyrethroids, organophosphate and organochlorine). The immature stages were reared to the adult stage at the insectary unit of integrated vector management and the first-generation progeny (F1) were exposed to WHO insecticide-impregnated papers. The treatments used for the mosquitoes consist of carbamates (0.1% bendiocarb), organophosphates (0.25% pirimiphos-methyl), pyrethroids (0.05% deltamethrin, permethrin 0.75 %) and organochlorines (4% DDT). Ae. aegypti is susceptible to deltamethrin, permethrin and pirimiphos methyl (100%) mortality, whereas highly resistant to DDT 29 % and low resistant to bendiocarb 82%. Moreover, *An. gambiae complex* is susceptible to organophosphate group 100% mortality and resistant to pyrethroids (deltamethrin 42%, permethrin 83 %) and DDT 79%) table 1. *Culex* sp resists all insecticide classes except pirimiphos methyl 98%.

Keywords: Insecticide resistant, *Ae, aegypti, An. gambiae complex, Culex sp* and El geneina town, Sudan

1. Introduction
Integrated vector management is a core component and effective strategy recommended by WHO global Malaria program to prevent vector-borne disease when implemented properly [1]. The existence of vector resistance to insecticides is becoming a major threat to malaria and vector-borne disease prevention measures [2, 3, 4]. This is the common challenge facing vector-borne disease control programs in Africa that can have a negative impact on fighting arboviral illnesses [5]. Moreover, most of the mosquito population developed insecticide resistance to organic insecticides that were used during the 20th century [3]. Further, monitoring of insecticide resistance is a core function of Malaria Control Programs and plays an important role in identifying the resistance mechanism [6, 7]. The use of long-lasting insecticidal nets (LLINs) and indoor residual spraying (IRS) are the main components of most malaria prevention and control strategies. Four classes of insecticide groups are used in IRS but pyrethroids are only used in LLIN [7, 8].
In Sudan resistance to DDT and pyrethroids was detected with strong seasonal variations evident in Anopheles arabiensis [8]. Therefore, the insecticide resistance monitoring was implemented at a two-year interval, later was applied on a yearly basis. In west Darfur, the application of space spray and LLIN has increased widely in two previous decades, using pyrethroids to control malaria and viral hemorrhagic fever that required regular monitoring of insecticide resistance of *An. gambiae complex, Ae. aegypti* and *Culex sp.* Furthermore, despite these efforts, the data on susceptibility status are poorly documented. In the present study we aim to identify the susceptibility status of *An. gambiae complex, Ae. aegypti* and *Culex* sp to four insecticide classes. This was carried out as strong evidence for an insecticide resistance management strategy as well as selecting appropriate, effective, and efficient insecticides in west Darfur state to eradicate Malaria and viral hemorrhagic fever vectors.

2. Methods

2.1 Study area

West Darfur State is in the western part of Sudan between 110 - 150 N and 220 - 250 E. It is located across two different climatic zones, savannah with high rainfall on the southern side and the semi desert in the north. The state is mainly flat but interrupted in some areas by small hills and seasonal valleys. The state is occupied by different soil types in different parts; however, most areas are dominated by sandy soil. The climate prevailing in the state is tropical continental; temperatures range in the area between 10 - 400C. The maximum temperature reaches 40o C and above in summer (March to June), with a mean monthly temperature of around 35 o C, and the minimum temperature is 10-250C in winter (December to February). The rainfall varied between 200 - 700 mm in the area according to the different climatic zones prevailing in the state.

2.2 Study sites

El geneina town is in the savannah zone with high rainfall at open border with Aderi town, Republic of Tchad (distance less than 30 km) with continuous cross movement between inhabitance for purposes of marketing and health care services. The area is inhabited by 408,748 people where 125.431 (30.6%) are IDPs. Mosquito collection was carried out from four areas around Kaga valley Beult and areas named: Omdoin, Ardamata, Elgabel, Almadaris and El Shati figure 1.

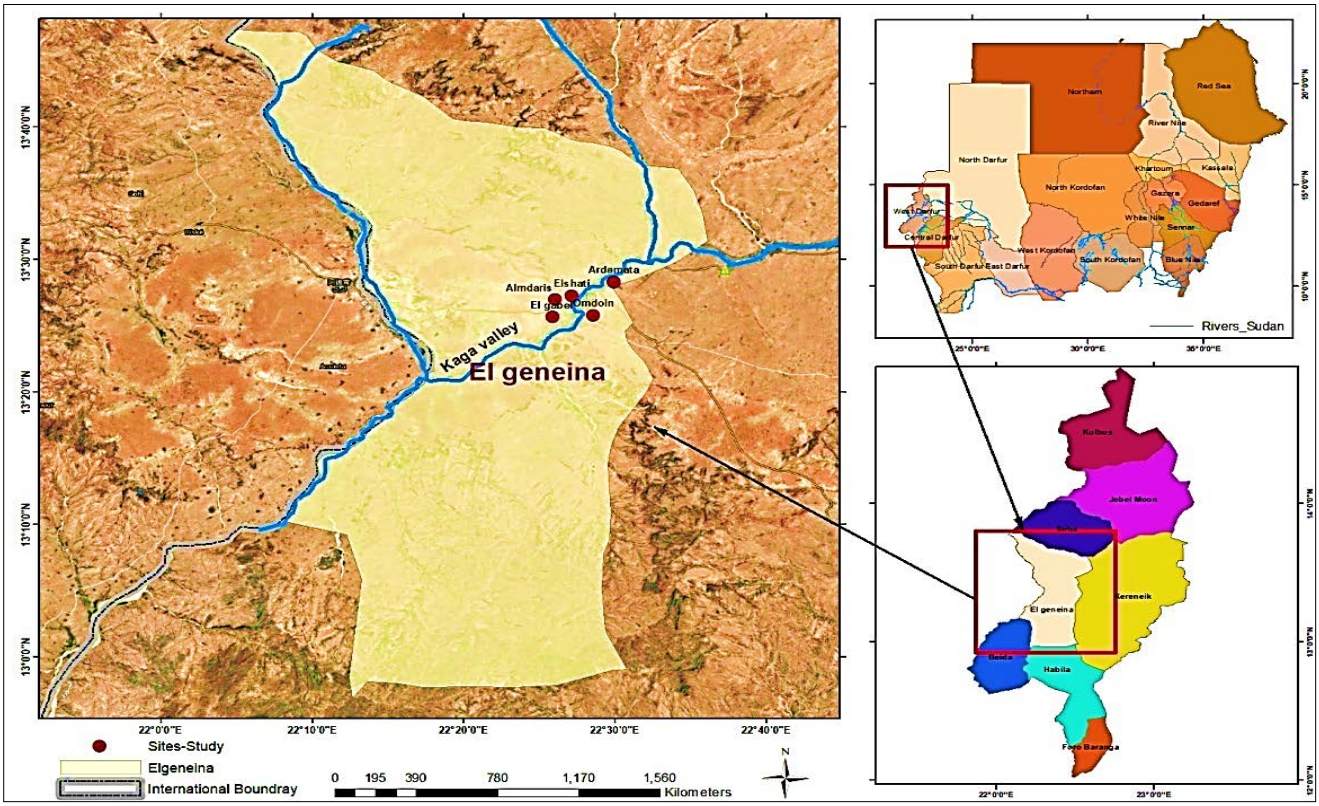

Fig 1: shows study sites of Insecticides Susceptibility status, Elgeneina, Sudan.

2.3 Study population

3ed,4th larval instars and pupae of *Ae. aegypti, An. gambiae complex and Culex sp* as well as female adult stage.

2.4 mosquito collection and rearing

The collection of *Anopheles* and *culex* immature stages was conducted using white dishes, fine mesh sieves, dippers, pasture pipettes, labeled vials, marker pain, forms as well as GPS to record information on types of breeding sites and geographical location. Moreover, the dips were transferred into white dishes; the larvae and pupae were sorted from predators and then transferred into labeled vials too. All collected vials were saved in a box covered with a wet towel. Nevertheless, *Aedes* aquatic stages were collected using equipment mentioned by [9]. The collection was carried out through house inspection. All positive containers inside houses were sorted from the larvae and pupae of *Aedes aegypti*, transferred to labeled vials into a small box covered with wet towels before being transferred to the laboratory. Mosquito rearing was carried out in a state of insecurity under conditions as mentioned in [10]. Firstly, we transferred collected aquatic stages from the field into specific white dishes with separation to (*Anopheles*, *Culex* and *Aedes*), water from the field was replaced by clean water and follows feeding with rice powder with regular monitoring of relative humidity, temperature, feeding status as well as developmental progress. When 4th larval instars were converted into pupae, we transferred them into labeled plastic cups and put them in cages. An emerging adult feeding on 10 % sugar solution, 3 - 4 days of age, with good physiological and morphological status was prepared for the test procedures.

2.5 Sample size

A total of 150 adult female mosquitoes derived from larvae and adults were selected to conduct a single set of WHO insecticide susceptibility tests. Of these, 100 were specified for exposure and 50 for control, 25 mosquitos in each replicate. We applied these numbers for each single test for

deferent insecticide classes. When it is not possible to test this number of mosquitoes on a single day, tests are conducted over a few days until this number is reached, if those control tests are run in parallel. Physiological and physical status (feed on 10% sugar solution) of the target population were considered during selection (female age 3-4 days) older than this were excluded.

2.6 species identification

Morphological identification was carried out at species level (*Ae. aegypti*, *An. gambiae complex*) and *Culex* at genus level using [11, 12]. After completing the test, all specimens were saved over silica gel with separate classification to resist and susceptible to advanced investigation.

2.7 test condition and protocols

Therefore, temperature and humidity need to be controlled during the test and holding periods. Ideally, tests were carried out at 25 °C ± 5 °C and 75% ± 10% relative humidity. During the exposure and holding periods, both the temperature and relative humidity were monitored using a hydrometer and recorded in standard form, with the maximum and minimum values recorded at the start of the exposure period and again at the end of the holding period. The impregnated papers were used less than 6 times for every single test. After the end of the tests, they were re-charged into the plastic box specified for them and saved under room temperature in a darkened cupboard. The tests were conducted as the following [10].

2.9 Insecticide susceptibility test (WHO bioassay tests)

Mortality resulting from contact with insecticide-treated filter papers was measured using WHO test kits [10]. The tests were carried out using malathion 5 %, deltamethrin 0.05 %, permethrin 0.75 %, pirimiphos methyl 0.25 %, bendiocarb 0.1 %, and DDT 4 %. Insecticide-impregnated papers were obtained from the Malaysian WHO Collaborating Center at standard concentrations for determining resistance of adult mosquitoes. Four batches of 25 unfed females were exposed to impregnated papers for 1h. The number of knock-down mosquitoes was recorded after 10, 15, 20, 30, 40, 50 and 60 minutes. Tests with untreated papers that served as control were run in parallel. At the end of the exposure period, mosquitoes were transferred into tubes with untreated white filter papers (known as holding tubes) and allowed a 24-h recovery period. All mosquitoes were provided with 10 % glucose water during the 24-h recovery period. The mmortality rate was recorded after 24 h.

2.9 Data collection and analysis

Data on ecology was collected using standard form and GPS, analysis was carried out using an Excel program, and a parametric test was calculated (mean + standard error as well as P value). The mortality of the test sample is calculated by summing the number of dead mosquitoes across all exposure replicates and then expressing this as a percentage of the total number of exposed mosquitoes: Total number of dead mosquitoes Observed mortality = total number of dead mosquitoes / Total sample size × 100. The control mortality was re corrected using Abbott's formula in one test when the percentage of mortality was less than 20% as follow: corrected mortality =

(% observed mortality - % control mortality) / (100 - % control mortality) × 100.

3. Results

Ae. aegypti is susceptible to deltamethrin, permethrin and primiphos methyl (100%) mortality, whereas highly resistant to DDT 29 % and lower resistant to at 82%. Moreover, *An. gambiae complex* is susceptible to organophosphate group 100% mortality and resistant to pyrethroids (deltamethrin 42%, permethrin 83 %) and DDT 79%) table 1. *Culex sp* resists all insecticide classes except primiphos methyl 98% table2.

Table 3: mean and standard error mortality and percentage of *Ae. aegypti* and *An. gambiae complex*, El geneina, Sudan 2018

Insecticides	*Ae. aegypti*						*An. gambiae complex*					
	No. exposed	% mortality	Mean ± SE	No of control	% mortality	Mean ± SE	No. exposed	% mortality	Mean ± SE	No of control	% mortality	Mean ± SE
DDT	100	29	11.6 ±4.5	50	0	0 (0)	100	79	31.6 ± 11.9	50	2	1.0 ± 1.0
Deltamethrin	100	100	40.0±15.0	50	0	0 (0)	100	42	16.8 ± 6.8	50	1	0.5 ± 0.5
Permethrin	100	100	40.0±15.0	50	0	0 (0)	100	83	33.2 ± 12.5	50	1	0.5 ± 0.5
Pirimimphos methyl	100	100	40.0±15.0	50	0	0 (0)	100	100	40.0± 15	50	0	0 (0)
Bendiocarb	100	82	32.8 ± 12.3	50	0	0 (0)	100	100	40.0± 15	50	4	2 ± 2

Table 4: mean and standard error mortality and percentage of *Culex sp*, El geneina, Sudan 2018

Insecticides	*Culex sp*					
	No. exposed	% mortality	Mean ± SE	No of control	% mortality	Mean ± SE
DDT	100	24	30.6 ± 11.4	50	1	0.5 ± 0.5
Deltamethrin	100	67	34.8 ± 13.0	50	6	3 ± 2.8
Permethrin	100	74	29.6 ± 11.3	50	1	0.5 ± 0.5
Pirimimphos methyl	100	98	39.2 ± 14.7	50	2	1.0 ± 1.0
Bendiocarb	100	88	35.2 ± 13.2	50	2	1.0 ± 1.0

4. Discussion

Monitoring of insecticide resistance is a core function of Malaria Control Programs and plays an important role in identifying the resistance mechanism [6, 7]. In Sudan, resistance to DDT and pyrethroids was detected with strong seasonal variations evident in *Anopheles arabiensis* [8]. In west Darfur, the application of space spray and LLIN has increased widely in two previous decades, using pyrethroids to control malaria and viral hemorrhagic fever that required regular monitoring of insecticide resistance *of An. gambiae*

complex, Ae. aegypti and *Culex sp.* According to WHO insecticide susceptibility bioassay, the results of this study showed that *Ae. aegypti* developed resistance to DDT and Bendiocarb by 29% and 82%, respectively. The possibility of resistance to DDT may refer to the historical use of this class in Malaria control as well as mis-use in the agricultural field. These findings are consistent with similar study findings conducted in Awka, Nigeria [5, 13, 14]. The development of resistance to Bendiocarb that occurred may be due to agricultural uses, because this class was not applied this class in the study area previously. *Ae. aegypti* susceptible to Deltamethrin, Permethrin and pirimiphos methyl all recorded 100% mortality. This is agreed with results obtained from Nigeria that revealed the susceptibility of *Ae. aegypti* to Pirimiphos methyl 98.75% and deltamethrin 100% and is consistent with findings obtained from Malaysia [13], in contrast with studies carried out by [15, 16]. The findings showed the development of *Ae. aegypti* resistance to one pyrethroids class, and the findings of [17, 18, 19, 20], highlighted that all field-collected *Aedes aegypti* strains were resistant to the four pyrethroids tested, except one area. *Anopheles gambiae complexes* susceptible to the organophosphate group (Pirimiphos methyl and Bendiocarb) were both recorded at a 100% mortality rate. This, in consistent with findings obtained from Khartoum, revealed that *An. arabiensis* was susceptible to bendiocarb at 98.1% [2]. Also, in agreement with findings from Madagascar [21], and other results obtained from Mozambique [22] and [23] from Tanzania recorded 100% mortality in bendiocarb and findings obtained by [8] from Gezira state. Whereas resist to permethrin, deltamethrin and DDT, 83%, 42% and 79% respectively. These findings, in agreement with recent studies carried out in central Sudan, highlighted the presence of multiple insecticides' resistance to permethrin, deltamethrin and DDT in deferent seasons [8, 24, 25]. Similarly, *An. arabiensis* showed insecticide resistance to deltamethrin (64%), permethrin (77%) and lambda cyhalothrin (42%) [23]. Moreover, An. gambiae complex was susceptible to deltamethrin, DDT and bendiocarb in Mozambique, Madagascar, and Sudan [2, 21, 22]. The variation in resistance of *An. gambiae complex* to different insecticide classes may refer to long-term exposure to deferent insecticide groups in deferent ways, such as exposure to pyrethroids (permethrin) when mosquitoes rest at the surface of long-lasting insecticidal nets as well as treated walls. Moreover, wide uses of insecticides during contentment of dengue and chikungunya outbreaks in previous decades may have played an important role in developing resistance due to abuse of insecticides and programs depend mainly on chemical control besides water source reduction. *Culex* sp resists all insecticide classes except pirimiphos methyl 98%. Therefore, DDT recorded 24% mortality, permethrin 74%, deltamethrin 67% and bendiocarb 88%. Similarly, study findings from Sri Lanka showed that existence of genetic resistance of *Cx. quinquefasciatus* against pyrethroids [26]. Moreover, *Cx. quinquefasciatus* was also observed to resist all classes in deferent areas in Nigeria and Thailand [27, 28]. Similar studies showed high levels of resistance were recorded with a mortality rate after 24 hours for DDT ranging from 20% to 32%, while permethrin ranges from 14% to 36% in *Cx. quinquefasciatus* [29, 30, 31]. The present study represents the first study in the area to monitor the susceptibility of *Culex* sp to insecticides. The findings showed that resistance of *Culex* species to all groups and classes of insecticides except pirimiphos methyl. The variation in susceptibility of *Ae. aegypti, An. gambiae complex* and *Culex* may refer to deferent methods, time of application, resting habitat (indoor or outdoor) as well as feeding behavior (endo or exo - phagic). However, highly resistant, less than 90%, are recorded in deferent insecticide classes and vectors that do not need more conformation by PCR [10]. There is therefore an urgent need to implement a proper insecticide resistance management strategy, in particular the use of pyrethroids in the control of malaria vectors.

5. Conclusion

The findings showed the development of *Ae. aegypti* resistance to one pyrethroids class, and the findings of [17, 18, 19, 20] highlighted that all field-collected *Aedes aegypti* strains were resistant to the four pyrethroids tested, except one area. *Anopheles gambiae complexes* susceptible to the organophosphate group (Pirimiphos methyl and Bendiocarb) were both recorded at a 100% mortality rate.

Reference

1. Rochlin I, Ninivaggi DV, Hutchinson ML, Farajollahi A. Climate change and range of expansion of the Asian tiger mosquito (Aede salbopictus) in north-eastern USA; Implication for public health practitioners. PLOS ONE. 2013;8:e60874.
2. Seidahmed MA, Abdelmajed MS, Mustafa AP, Mnzava AP. Insecticide susceptibility status of the malaria vector Anopheles arabiensis in Khartoum city, Sudan: differences between urban and periurban areas. EMHJ. 2012;18(7).
3. Singh RK, Mittal PK, Kumar G, Dhiman RC. Insecticide susceptibility status of *Aedes aegypti* and *Anopheles stephensi* larvae against temephos in Delhi, India. Int J Mosq Res. 2014;1(3):69-73.
4. Nwankwo EN, Ononye IP, Ogbonna CU, Ezihe EK, Onwude CO, Nwangwu UC. Insecticide Susceptibility Status of *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae) in Awka South Local Government Area, Anambra State, Nigeria. Entomol Ornithol Herpetol. 2019;8(3):1000220.
5. Poopathi S, Tyagi BK. The challenge of mosquito control strategies: From primordial to molecular approaches. Biotechnol Mol Biol Rev. 2006;1(2):51-56.
6. World Health Organization. World Malaria Report 2010. Geneva: WHO; 2012.
7. World Health Organization. Global Plan for Insecticide Resistance Management in malaria vectors. Geneva: WHO; 2012.
8. Abdalla H, Wilding CS, Nardini L, Pignatelli P, Koekemoer LL, Ranson H, *et al*. Insecticide resistance in *Anopheles arabiensis* in Sudan: temporal trends and underlying mechanisms. Parasit Vectors. 2014;7:213.
9. Focks DA. A review of entomological sampling methods and indicators for dengue vectors (No. TDR/IDE/DEN/03.1). Geneva: World Health Organization; 2004.
10. World Health Organization. Test procedures for insecticide resistance monitoring in malaria vector mosquitoes, Second edition. Geneva: WHO; 2018.
11. Edwards FW. Mosquitoes of the Ethiopian Region. HI.-Culicine Adults and Pupae. Mosquitoes of the Ethiopian Region. HI. Culicine Adults and Pupae. 1941;499(11).

12. Lewis DJ. The Stegomyia mosquitoes of the AngloEgyptian Sudan. Ann Trop Med Parasitol. 1953;47:51-61.
13. Hadura AH, Zairi J, Ranson H, McCall P. Pyrethroid and Organophosphate susceptibility status of *Aedes aegypti* (Linnaeus) and *Aedes albopictus* (Skuse) in Penang, Malaysia. Int J Entomol Res. 2015;3(3):91-95.
14. Kamgang B, Marcombe S, Chandre F, Nchoutpouen E, Nwane P, Etang J, *et al*. Insecticide susceptibility of *Aedes aegypti* and *Aedes albopictus* in Central Africa. Parasit Vectors. 2011;4:79.
15. Ramadhani RZ, Ishak H, Zulkifli A, Rusman AD, Haniarti, Usman, *et al*. Susceptibility status of *Aedes aegypti*, *Aedes albopictus*, *Culex quinquefasciatus* and *Anopheles subpictus* to insecticide cypermethrin. Earth and Environmental Science. 2020;575:012003.
16. Ikawati B, Sunaryo S, Widiastuti D. Peta status kerentanan *Aedes aegypti* (Linn.) terhadap insektisida cypermethrin dan malathion di Jawa Tengah. Aspirator - J Vectorborne Dis Stud. 2015;7:23-8.
17. Siti-Futri FF, Rosilawati R, Wan KL, Cheong YL, Nazni WA, Lee HL. Status of pyrethroid resistance in *Aedes (Stegomyia) aegypti* (Linneaus) from dengue hotspots in Klang Valley, Malaysia. [no journal info or page number].
18. Wan-Norafikah O, Nazni WA, Lee HL, Zainol-Ariffin P, Sofian-Azirun M. Permethrin resistance in *Aedes aegypti* (Linnaeus) collected from Kuala Lumpur, Malaysia. J AsiaPacific Entomol. 2010;13(3):175-182.
19. Loke SR, Wei Ann AT, Ahmad NW, Azirun MS. Insecticide susceptibility status of field collected *Aedes (Stegomyia) aegypti* (L.) from a dengue endemic site in Shah Alam, Selangor, Malaysia. Southeast Asian J Trop Med Public Health. 2012;43(1):34.
20. Ishak IH, Jaal Z, Ranson H, Wondji CS. Contrasting patterns of insecticide resistance and knockdown resistance (kdr) in the dengue vectors *Aedes aegypti* and *Aedes albopictus* from Malaysia. Parasit Vectors. 2015;8(1):181.
21. Randriamaherijaona S, Velonirina HJ, Boyer S. Susceptibility status of *Anopheles arabiensis* (Diptera: Culicidae) commonly used as biological materials for evaluations of malaria vector control tools in Madagascar. Malar J. 2016;15:338.
22. Casemiro S, Coleman M, Hemingway J, Sharp B. Insecticide Resistance in *Anopheles arabiensis* and *Anopheles gambiae* from Mozambique. J Med Entomol. 2006;43(2).
23. Lwetoijera DW, Harris C, Kiware SS, Dongus S, Devine GJ, McCall PJ, *et al*. Increasing role of Anopheles funestus and *Anopheles arabiensis* in malaria transmission in the Kilombero Valley, Tanzania. Malar J. 2014;13:331.
24. Abdalla H, Matambo TS, Koekemoer LL, Mnzava AP, Hunt RH, Coetzee M. Insecticide susceptibility and vector status of natural populations of *Anopheles arabiensis* from Sudan. Trans Roy Soc Trop Med Hyg. 2008;102:263-271.
25. Matambo TS, Abdalla H, Brooke BD, Koekemoer LL, Mnzava A, Hunt RH, *et al*. Insecticide resistance in the malarial mosquito *Anopheles arabiensis* and association with the kdr mutation. Med Vet Entomol. 2007;21:97-102.
26. Geethika K, Chandrasiri PK, Fernando SD, De Silva NK. Insecticide resistance and molecular characterization of knockdown resistance (kdr) in *Culex quinquefasciatus* mosquitoes in Sri Lanka. J Vector Ecol. 2020;45(2).
27. Lawal N, Idoko AS, Hadiza A, Abdulqadir AI. Insecticide susceptibility bioassay in *Culex quinquefasciatus* vector of lymphatic filariasis from Sahel Savannah region of Northwest Nigeria. Int J Mosq Res. 2020;7(4):01-05.
28. Sunaiyana S, Pungasem P, Kasin S. "Detection of insecticides resistance status in *Culex quinquefasciatus* and *Aedes aegypti* to four major groups of insecticides". Trop Biomed. 2006;23(1):97-101.
29. Agbor OV, Idowu TE, Fagbohun KI, Oyeniyi AT, Jimoh RT, Otubanjo AO. Molecular Identification and Insecticide Resistance Status of Culex mosquitoes collected from blocked drainages in Lagos State, Nigeria. Pan African Journal of Life Sciences. 2020;4(1):1-6.
30. Fagbohun IK, Oyeniyi TA, Idowu TE, Otubanjo OA, Awolola ST. Cytochrome P450 Monooxygenase and Resistance Phenotype in DDT and Deltamethrin-Resistant *Anopheles gambiae* (Diptera: Culicidae) and *Culex quinquefasciatus* in Kosofe, Lagos, Nigeria. J Med Entomol. 2019;56(7):817-821.
31. Oduola AO, Obembe A, Adelaja OJ, Ande AT. Surveillance and insecticide susceptibility status of Culicine mosquitoes in selected communities utilizing long-lasting insecticidal nets in Kwara State, Nigeria. Anim Res Int [Internet]. 2016;13(3):2483-2491.