

Larval density and physicochemical properties of three different breeding habitats of *Anopheles* mosquitoes in Sudan savannah region of Jigawa State, Nigeria

Asma'u Mahe^{1,2}, Adamu Jibril Alhassan¹, Chimaobi James Ononamadu³,
Nura Lawal⁴, Sadiya Ahmad Bichi⁵, Sani Ado Haruna⁶,
Firdausi Abdulkadir Sani⁶, Abdullahi Abdulkadir Imam^{1*}

¹Department of Biochemistry,
Faculty of Basic Medical Sciences,
Bayero University, Kano, Nigeria.

²Department of Biochemistry,
Faculty of Science,
Federal University, Dutse,
Jigawa State, Nigeria.

³Department of Biochemistry and Forensic Science,
Nigeria Police Academy,
Wudil, Nigeria.

⁴Department of Biochemistry,
Faculty of Science,
Federal University, Dutsinma,
Katsina State, Nigeria.

⁵Department of Biochemistry,
Faculty of Science,
Kano University of Science and Technology,
Wudil, Kano State, Nigeria.

⁶Department of Biochemistry,
Faculty of Science,
Yusuf Maitama Sule University, Kano,
Kano State, Nigeria.

E-mail: aaaimam.bch@buk.edu.ng

Abstract

Mosquitoes are tiny insects that can serve as vectors for numerous infectious diseases like malaria. Malaria is considered as one of the major causes of mortality, loss of productivity and a major contributor of poverty in the African continent. In 2020, most global malaria cases (94%) were recorded in the Africa Region. In Nigeria, about 97% of the estimated populations are at risk of malaria, with up to 27% and 24% of Africa and global malaria deaths respectively in 2018. Malaria control programmes in Africa have challenge of rising insecticide resistance in the main anopheline vectors, this affects primary malaria vector control interventions. In Africa, the dominant mosquito species that transmit

*Author for Correspondence

malaria parasites are mainly Anopheles gambiae s.s. A key control strategy against major mosquito-borne diseases involves targeting mosquito vectors to disrupt the transmission of diseases. Environmental changes can alter the genetic structure, protein profiles and enzymes of mosquitoes leading to increase in insecticide resistance. Knowing the ecology, spatial distribution of mosquito larvae, and some environmental features like physicochemical factors are important in tackling insecticide resistance. Water and larval samples were collected from three Anopheles mosquito breeding sites in August, September and October, 2019. Physicochemical parameters, larval densities and morphological specie identifications were determined. Results from this study reveal variation in levels of each studied parameter according to the site of the study. These variations can be attributed to differences in activities that occur in each site of the study.. Correlation studies indicated a significantly higher larval density in agricultural site relative to industrial and residential sites, this was by a magnitude of 10.65 and 41.30 respectively. Anopheles gambiae s.s. complex was found to be predominant in all the three study sites. These physicochemical parameters can either have negative or positive effect on mosquito biology depending on their levels, hence can affect vector control measures making it significant in terms of vector control programmes.

Keywords: *Anopheles* mosquitoes, Malaria, physicochemical parameters, larval density

INTRODUCTION

Mosquitoes are tiny insects that belong to *Arthropoda*, *Hexapoda*, *Insecta*, *Diptera*, *Nematocera*, and *Culicidae*, as phylum, superclass, class, order, suborder, and family respectively with subfamilies of *Anophelinae*, *Culicinae*, *Toxorhynchitinae* (Ilahi and Suleman, 2013). These species of mosquitoes serve as vectors for numerous infectious diseases, about 3,500 mosquito species were classified into 41 genera (Kilpatrick, 2011), this feature is relative to their abundance, diversity, vector capacity and recurrent infection (Njabo, 2013). Mosquitoes of *Anopheles*, *Culex* and *Aedes* genera are among the main vector species of protozoan, nematode and virus pathogens (Wilke and Marrelli, 2015). Mosquitoes transmit and vector diseases like malaria, yellow fever, dengue and some other diseases to millions of people annually (Noutchamae and Anumudu, 2009). In Africa, the dominant mosquito species that transmit malaria parasites are mainly *Anopheles gambiae s.s. complex* and *Anopheles funestus*. These species are widely spread over tropical and subtropical Africa (Coetzee *et al.*, 2013).

Malaria is one of the major causes of mortality, and loss of productivity in the African region. It can also result in strong negative effect on developmental stages in children that can lead to permanent disability to many that survive the disease (Knox *et al.*, 2014). Malaria is considered as a major contributor of poverty in Africa (Okorosobo *et al.*, 2011). About 228 and 229 million malaria cases were globally recorded in 2018 and 2019 respectively. Most of these cases (94%) were recorded in the Africa Region (WHO, 2021). In Nigeria, about 97% of the estimated populations are at risk of malaria, with up to 27% and 24% of Africa and global malaria deaths respectively (WHO, 2019). Six countries are responsible for more than half of all the global malaria cases, these countries include; Nigeria, Democratic Republic of Congo and Uganda with 25%, 12% and 5% of the global cases respectively (WHO, 2019). Malaria control programs in Africa have challenge of rising insecticide resistance in the main anopheline vectors, thus, affecting primary malaria vector control interventions (Knox *et al.*, 2014).

A key control strategy against major mosquito- borne diseases involves targeting mosquito vectors to disrupt the transmission of diseases (Niyang *et al.*, 2018). Long-term use of agrochemicals in agricultural practices alter the natural environment thereby leading to the selection of resistance in insect species (WHO, 2013; Alhassan *et al.*, 2015). Knowing the ecology and spatial distribution of mosquito larvae is significant in the effectiveness of vector control measures (Mereta *et al.*, 2013). Breeding site water parameters such as: pH,

temperature, ammonia, phosphate, sulphate, nitrate, nitrite, chloride, calcium e.t.c affect larval density of mosquitoes. Changes in these parameters of the breeding sites might result in positive or negative effects to the vectors. Temperature below 14°C and above 30°C decrease larval growth rate in most vector specie. Many mosquito larvae are naturally found in pH 3.3- 10.5, dissolved nitrogen content can negatively affect larval growth of some vectors (Liu *et al.*, 2012; Nikookar *et al.*, 2017). Environmental temperature changes can alter the genetic structure, protein profiles and enzymes of mosquitoes leading to increase in resistance (Soko *et al.*, 2015). Proper understanding of the nature of resistance is important in the management and control of malaria vectors (Nkya *et al.*, 2014). Monitoring and knowledge of environmental factors like physicochemical parameters are important in tackling insecticide resistance challenges (Ononamadu *et al.*, 2020). Liu *et al.*, 2012, Mereta *et al.*, 2013, and Nikookar *et al.*, 2017 studied physico-chemical characteristics of anopheline mosquito larval breeding habitats elsewhere as implications for control of malaria. Alhassan *et al.*, 2015, Safiyanu *et al.*, 2016, Safiyanu *et al.*, 2017, Safiyanu *et al.*, 2019 and Ononamadu *et al.*, 2020 reported insecticide resistance in *Anopheles* mosquitoes from areas of the same region as that of this study. Reports on the larval density and physicochemical parameters of mosquitoes' breeding sites of this study area is lacking. Hence the need to study these parameters of *Anopheles* mosquitoes' breeding sites of the study area.

MATERIALS AND METHOD

Study Area

The study was carried out in Sudan savannah region of Jigawa State. Three sites were studied; (1) Agricultural and (2) Industrial sites from Hadejia town (Latitude: 12°44'98"N, Longitude: 10°04'44"E) while (3) Residential site from Dutse town (Latitude: 11°75'62"N, Longitude: 9°33'90"E). The State has a total land area of approximately 22,410 km² and a density of 251.7 per km². It has coordinates of: 12° 00' N 9° 45' E between latitudes 11.00° N to 13.00° N and longitudes 8.00° E to 10.15° E (JGS, 2015). Occupation include: fishing, rice farming and establishment of irrigation based activities.

Sample Collection

Breeding sites water samples and *Anopheles* mosquito larvae were collected from randomly selected vegetation farms, choked gutters, water logged, marshy or swampy areas around each study site during rainy season (August, September and October, 2019). These sampling sites were found highly polluted with organic materials.

A dipper was used to obtain *Anopheles* larvae after screening for the presence of the larvae. *Anopheles* larvae were transferred along with breeding waters to the holding containers before searching for more. This procedure was done over and over until significant numbers of larvae were obtained for each period of the study. This procedure was done according to Robert *et al.*, (2002).

Parameters Determination

Twenty- seven (27) parameters were determined from mosquitoes' breeding site water samples obtained from the three study sites. These parameters were: pH, temperature, electrical conductivity (EC), turbidity, total dissolved solids (TDS), total suspended solids (TSS), dissolved oxygen (DO), carbonates, bicarbonates, ammonium, nitrates, phosphates, sulphates, nitrites, chlorides, potassium, sodium, calcium, magnesium, zinc, iron, copper, nickel, cobalt, lead, manganese, cadmium. They gave an insight to the kind of activities performed in the study sites.

DO (dissolved oxygen) was determined using portable DO meter in the laboratory after calibration. The probe was calibrated before use and distilled water was used to rinse the probe each time of use to avoid cross contamination. Total Suspended Solids (TSS) and Total Dissolved Solids (TDS) were done according to APHA (1985).

Physical analysis

Physical parameters including: pH, temperature, and electrical conductivity were determined by a portable multi-function water quality checker (Model Number: EZ- 9908) at each sampling site and the results were recorded.

Mineral elements Determination

Sulphate and nitrate were determined according to the method of APHA (1985). Chloride, bicarbonate and carbonate were measured using method of Trivedi and Goel (1986). For determination of the following: phosphate, potassium, magnesium, zinc, nickel, cadmium, iron and manganese, standard methods were used as described by American Public Health Association (APHA, 2005). Copper, cobalt, and lead content of the breeding site water samples were measured by atomic absorption spectrophotometry (AAS). Sodium and calcium were determined by flame photometry.

Digestion of water sample: Water samples were prepared (digested) prior to analysis. 100ml of well mixed water sample was transferred into a clean beaker; 5ml of conc. HNO₃ was added and slowly heated to boil on a hot plate. It was then allowed to cool and another 5ml of conc. HNO₃ was added. The beaker was covered with a watch glass and returned to the hot plate. A gentle refluxing action of the solution was set by increasing the temperature of the hot plate. Heating was continued with addition of acid as required until digestion was completed. About 1.2ml conc. HNO₃ was added to dissolve the residue. The residue was washed with distilled water and filtered to remove silicate and other insoluble material. The volume of the solution was adjusted to 100ml in a volumetric flask. A reagent blank determination was carried out. The digested solution was transferred to plastic bottles labelled accurately and were used for mineral determination (AAS and flame photometry).

Specie Identification (Morphological Species identification)

Anopheles mosquito larvae were identified based on spatial projections on the water surface being horizontally based. Morphological identification was performed according to the keys of (Gillies and Coetzee, 1987). Characteristics unique to all *Anopheles* mosquitoes were screened using a Zeiss ×10 light microscope.

Statistical Analysis

Larval density and physicochemical parameters were expressed as mean ± standard deviation and were analyzed by one-way ANOVA using SPSS Version 20 for comparison of variations between the sampling sites with *p* value <0.05 as significant. Pearson correlation, Linear (multiple) regression and ANOVA were used to study the relationship between larval densities and physicochemical properties, between larval densities and study sites.

RESULTS

Table 1 gives the physicochemical parameters and larval densities of water from agricultural, industrial and residential breeding sites of *Anopheles specie* in Hadejia and Dutse. Results from the table indicate varying levels of the assayed parameters in the study sites. Some of the observed variations in the parameters were found to be statistically significant.

Table 1: Physicochemical parameters and larval densities of *Anopheles* mosquitoes' breeding sites water samples from Agricultural, Industrial and Residential sites of Hadejia and Dutse

SITES	pH	TEMP (°C)	EC (µS/cm)	TURB. (NTU)	TDS (mg/L)	TSS (mg/L)	DO (mg/L)	Larval density/L
Agricultural site	7.77 ± 0.7a	28.57 ± 1.1a	223.00 ± 65a	30.00 ± 3.0a	119.26 ± 3.9a	0.12 ± 0.0a	4.76 ± 0.7a	73.33 ± 1.5a
Industrial site	6.18 ± 0.3b	30.13 ± 1.1a	216.00 ± 53a	62.20 ± 5.3b	62.23 ± 4.4b	0.28 ± 0.0a	4.06 ± 0.5a	62.67 ± 2.0b
Residential site	8.60 ± 0.5a	25.00 ± 1.5b	253.00 ± 83a	14.80 ± 1.8c	102.06 ± 3.7a	0.05 ± 0.0a	5.00 ± 0.8a	32.00 ± 2.6c

EC- Electrical Conductivity, TURB.- Turbidity, TDS- Total Dissolved Solid, TSS- Total Suspended Solid, DO- Dissolved Oxygen. Values were expressed as mean ± standard deviation. Statistical difference, ($p < 0.05$) using ANOVA and Turkey's HSD test, SPSS version 20. **Superscripts:** values bearing different letters down a column (across the sites) are statistically different ($p < 0.05$).

Table 2 indicates differences in levels of chemical parameters of water sample from agricultural, industrial and residential breeding sites of *Anopheles specie* in Hadejia and Dutse towns. Carbonate, ammonium, nitrate, phosphates, and sulphates varied significantly in agricultural site compared to industrial and residential sites.

Table 2: Chemical parameters of *Anopheles* mosquitoes' breeding sites water samples from Agricultural, Industrial and Residential sites of Hadejia and Dutse

SITES	CO ₃ ²⁻ (mg/L)	HCO ₃ ⁻ (mg/L)	NH ₄ ⁺ (mg/L)	NO ₃ ⁻ (mg/L)	PO ₄ ³⁻ (mg/L)	SO ₄ ²⁻ (mg/L)	NO ₂ ⁻ (mg/L)
Agricultural	28.02 ± 4.8a	50.16 ± 6.2a	3.17 ± 0.3a	11.55 ± 0.9a	98.20 ± 1.5a	218.74 ± 3.8a	3.21 ± 0.1a
Industrial	32.46 ± 3.8b	50.27 ± 5.9a	4.04 ± 0.1b	14.70 ± 0.5b	148.64 ± 5.0b	317.64 ± 7.0b	4.05 ± 0.4a
Residential	30.53 ± 2.5b	50.68 ± 4.1a	3.91 ± 0.2b	14.22 ± 0.8b	122.79 ± 2.6c	279.64 ± 3.2b	3.97 ± 0.5a

Values were expressed as mean ± standard deviation. Statistical difference, ($p < 0.05$) using ANOVA and Turkey's HSD test, SPSS version 20. **Superscripts:** values bearing different letters down a column (across the sites) are statistically different ($p < 0.05$).

Table 3 shows elemental parameters of water from Agricultural, Industrial and Residential breeding sites of *Anopheles specie* in Hadejia and Dutse towns. Results gave differences with some elements; chloride, sodium, calcium and magnesium differing significantly agricultural site compared to industrial and residential sites.

Table 3: Elemental parameters of *Anopheles* mosquitoes' breeding sites water samples from Agricultural, Industrial and Residential sites of Hadejia and Dutse

SITES	Cl ⁻ (mg/L)	K ⁺ (mg/L)	Na ⁺ (mg/L)	Ca ²⁺ (mg/L)	Mg ²⁺ (mg/L)	Zn ²⁺ (mg/L)	Fe ²⁺ (mg/L)	Cu ²⁺ (mg/L)	Ni ²⁺ (mg/L)	Co ²⁺ (mg/L)	Pb ²⁺ (mg/L)	Mn ²⁺ (mg/L)	Cd ²⁺ (mg/L)
-------	------------------------	-----------------------	------------------------	-------------------------	-------------------------	-------------------------	-------------------------	-------------------------	-------------------------	-------------------------	-------------------------	-------------------------	-------------------------

Larval density and physicochemical properties of three different breeding habitats of *Anopheles* mosquitoes in Sudan savannah region of Jigawa State, Nigeria

Agricultural	29.66 ± 1.1a	9.04 ± 1.6a	20.17 ± 10.7a	26.04 ± 3.5a	3.80 ± 0.7a	0.06 ± 0.0a	0.03 ± 0.0a	0.004 ± 0.0a	0.004 ± 0.0a	0.020 ± 0.0a	0.007 ± 0.0a	0.002 ± 0.0a	0.237 ± 0.0a
Industrial	34.90 ± 1.6b	11.07 ± 2.4a	8.47 ± 3.8b	15.80 ± 1.4b	1.81 ± 1.2b	0.06 ± 0.0a	0.09 ± 0.1a	0.004 ± 0.0a	0.004 ± 0.0a	0.020 ± 0.0a	0.007 ± 0.0a	0.001 ± 0.0a	0.210 ± 0.0a
Residential	35.93 ± 1.6b	10.94 ± 2.5a	12.50 ± 7.7b	41.40 ± 5.4c	2.34 ± 1.3b	0.06 ± 0.0a	0.01 ± 0.0a	0.012 ± 0.0a	0.003 ± 0.0a	0.020 ± 0.0a	0.007 ± 0.0a	0.00 ± 0.0a	0.301 ± 0.0b

Values were expressed as mean ± standard deviation. Statistical difference, ($p < 0.05$) using ANOVA and Turkey's HSD test, SPSS version 20. **Superscripts:** values bearing different letters down a column (across the sites) are statistically different ($p < 0.05$)

Table 4 and 5 show correlation studies between *Anopheles* mosquito larval densities with some of their breeding sites' physicochemical parameters and correlation between *Anopheles* mosquito larval densities and the three study sites respectively. Table 5 also indicated that moving from agricultural site to either industrial or residential sites is associated with a decrease in larval density by a magnitude of 10.65 and 41.30 respectively.

Table 4: Correlation between *Anopheles* mosquito larval densities and some physicochemical parameters of their breeding sites

Larval density	Correlation Coefficient	t- value	p- value
Cadmium (mg/L)	8.65	0.40	0.69
Calcium (mg/L)	-0.06	1.32	0.19
Nitrite (mg/L)	24.12	8.54	0.00

Table 5: Correlation between *Anopheles* mosquito larval densities and the three breeding study sites

Sites	Mean ± Standard Deviation	Correlation Coefficient	p- value
Agricultural	73.35 ± 1.30 ^a	Ref	-
Industrial	62.70 ± 1.78 ^b	- 10.65	0.00
Residential	32.05 ± 2.26 ^c	- 41.30	0.00

Table 6 is on specie composition of reared adult *Anopheles* mosquitoes from the three study sites in Sudan Savannah, Northwestern Nigeria based on morphological specie identification. The table shows abundance of *An. gambiae* s.s complex in the three study sites.

Table 6: Specie composition of adult *Anopheles* mosquitoes based on morphological specie identification from the three study sites in Sudan Savannah, Northwestern Nigeria

Study Sites	<i>Anopheles</i> Species	
	<i>An. gambiae</i> s.s complex	<i>An. pharoensis</i>
Agricultural	100%	-
Industrial	100%	-
Residential	96%	4%

DISCUSSION

This study was carried out in three distinct sites namely: agricultural, industrial and residential sites in Sudan savannah region of Jigawa State. Hadejia town was taken for agricultural and industrial sites while Dutse town for residential site. Table 1 showed physicochemical parameters and larval densities of breeding sites water samples from the three study sites. The pH of industrial mosquitoes' breeding site was found to be significantly lower than that of agricultural and residential sites. Temperature variations was also observed, the temperature of residential was significantly lower than the temperatures of the other two sites. These findings can be attributed to differences in the kind of activities occurring in the various study sites. Turbidity, Total dissolved solids (TDS), larval density also varies significantly between the study sites while electrical conductivity (EC), total suspended solids (TSS) and dissolved oxygen (DO) varies non significantly between the breeding sites. Nikookar *et al.*, (2017) reported the significance of breeding site water parameters in development and oviposition of mosquitoes. They also reported that changes in the parameters in larval breeding sites can affect mosquito biology positively or negatively. These parameters can be used as source of energy that help in growth of micro-organisms that serve as food for larvae and also help in oviposition and egg hatching (Kibuthu *et al.*, 2016). Table 2 showed significantly lower carbonate, ammonium, nitrate, phosphate, and sulphate in agricultural site compared to industrial and residential mosquito breeding sites. Table 3 gave the elemental parameters of water from the three breeding sites of *Anopheles* mosquitoes indicating varying levels of each studied element depending on the site of the study, these variations can also be attributed to differences in activities that occur in each site of the study. Ononamadu *et al.* (2020) reported significantly higher levels of some physicochemical parameters determined in industrial area compared to residential area of their study. Environmental factors due to human activities could be a key factor in conferring resistance in malaria vector (Alhassan *et al.*, 2015).

Table 4 presented correlation between *Anopheles* mosquito larval density and some of their breeding sites' physicochemical parameters, parameters that gave significant and high correlation coefficient were presented in the table. The table showed an inverse relationship between calcium level and larval density while increase in nitrite and cadmium levels are directly associated with increase in larval density. A statistically significant relationship exists between nitrite and larval density. Table 5 gave correlation between *Anopheles* mosquito larval density and the three study sites. The table indicated a significantly higher larval density in agricultural site relative to industrial and residential sites. It also indicated that moving from agricultural site to either industrial or residential sites is associated with decrease in larval density by a magnitude of 10.65 or 41.30 respectively. Larval conditions specifically larval density was reported to have both genotypic and phenotypic effects on some vector populations (Grossman *et al.*, 2018). Variations in levels of physicochemical parameters can have direct implications to vector control. These parameters can either have negative or positive effect on mosquito biology depending on their levels, hence can affect vector control measures. Detail of larval density and physicochemical parameters can help to

predict changes in case of environmental modifications due to either natural or artificial causes (Nikookar *et al.*, 2017).

Specie composition of adult *Anopheles* mosquitoes from the three study sites based on morphological specie identification was given in Table 6. Agricultural and industrial sites had 100% of *Anopheles gambiae s.s* complex while residential site had 96% as *Anopheles gambiae s.s* complex with 4% as *Anopheles pharoensis* which are secondary vectors of malaria. The dominant mosquito species in Africa that transmit malaria parasites are mainly *Anopheles gambiae s.s. complex* and *Anopheles funestus*, these species are widely spread over tropical and subtropical Africa (Coetzee *et al.*, 2013). This finding of abundance of a primary malaria vector in the three study areas can lead to increase in malaria infection in that area.

CONCLUSION

This study revealed variations in levels of physicochemical parameters and larval densities in the three studied sites: agricultural, industrial and residential breeding sites. Correlation studies indicated a statistically significant direct proportional relationship between nitrite and larval density, significantly higher larval density was observed in agricultural site relative to industrial and residential sites by a magnitude of 10.65 and 41.30 respectively. Also, one of the dominant mosquito species in Africa that transmit malaria parasites (*Anopheles gambiae s.s. complex*) was found to be predominant in all the three study sites, these findings are significant in vector control programmes.

Findings from this study can serve as an alarm to vector control programme of the study area because the study showed possible adaptation of *Anopheles* mosquitoes to their highly polluted breeding sites created by various human activities occurring in the breeding sites coupled with predominance of *Anopheles gambiae s.s. complex* in the area. These could result in high prevalence of malaria as a result of abundance of malaria vectors.

Current study provides baseline data and is regarded preliminary. It is recommended that more studies in the area should be done considering other parameters like; insecticide residue levels, residual hydrocarbons, chlorophyll, bacterial composition etc.

REFERENCES

- Alhassan, A. J., Sule, M. S., Dangambo M.A, Yayo, A. M., Safiyanu, M. and Sulaiman, D. (2015). Detoxification enzymes activities in DDT and bendiocarb resistant and susceptible malarial vector (*Anopheles gambiae*) breed in Auyo residential and irrigation sites Northwest Nigeria. *European Scientific Journal*. 11(9): 315- 326. ISSN: 1857 - 7881 (Print) e - ISSN 1857- 7431
- APHA (1985). Health Association, Washington, DC. Standard Methods for Examination of Water and Wastewater. 16th American Public
- APHA (2005) American Public Health Association. Standard Methods for the Examination of Water and Wastewater. 21st ed. Washington, DC: American Public Health Association.
- Chirebvu, E., and Chimbari, M.J. (2015). Characteristics of *Anopheles arabiensis* larval habitats in Tubu village, Botswana. *J Vector Ecol*. 2015; 40: 129±38. <https://doi.org/10.1111/jvec.12141> PMID: 26047193

- Coetzee, M., Hunt, R.H., Wilkerson, R., della Torre, A., Coulibaly, M.B., and Besansky, N.J. (2013). *Anopheles coluzzii* and *Anopheles amharicus*, new members of the *Anopheles gambiae* complex. *Zootaxa*, 3619:246–274.
- Gillies, M.T., and Coetzee, M. (1987). *A supplement to the Anophelinae of Africa south of the Sahara (Afrotropical region)*, Volume 55. South African Institute for medical research: Johannesburg.
- Grossman, M.K., Uc-Puc, V., Flores, A.E., Manrique-Saide, P.C., and Vazquez-Prokopec, G.M. (2018). Larval density mediates knockdown resistance to pyrethroid insecticides in adult *Aedes aegypti*. *Parasites & Vectors* (2018) 11:282 <https://doi.org/10.1186/s13071-018-2865-x>
- Ilahi, I., and Suleman, M. (2013). Species composition and relative abundance of mosquitoes in Swat, Pakistan. *Int J Innov App Stud*. 2013; 2(4):454-463.
- Jigawa State (JGS, 2015). A New World. [<http://www.jigawastate.gov.ng/contentpage.php?id=82>].
- Kibuthu, T.W., Njenga, S.M., Mbugua, A.K., and Muturi, E.J. (2016). Agricultural chemicals: life changer for mosquito vectors in agricultural landscapes? *Parasites & Vectors*. 2016; 9: 500. <https://doi.org/10.1186/s13071-016-1788-7> PMID: 27624456
- Kilpatrick, A. M. (2011). Globalization land use and the invasion of West Nile virus. *J Sci*; 334(6054):323-327.
- Knox, T. B., Juma, E. O., Ochomo, E. O, Jamet, H. P., Ndungo, L., Chege, P. (2014). An online tool for mapping insecticide resistance in major *Anopheles* vectors of human malaria parasites and review of resistance status for the Afrotropical region. *Parasites & Vectors*, 7:76 <http://www.parasitesandvectors.com/content/7/1/76>
- Liu, X.B., Liu, Q.Y., Guo, Y.H., Jiang, J.Y., Ren, D.S., and Zhou, G.C. (2012). Random repeated cross sectional study on breeding site characterization of *Anopheles sinensis* larvae in distinct villages of Yongcheng City, People's Republic of China. *Parasit Vectors*. 2012; 5:58. <https://doi.org/10.1186/1756-3305-5-58> PMID: 22444032
- Mereta, S.T., Yewhalaw, D., Boets, P., Ahmed, A., Duchateau, L., Speybroeck, N., Vanwambeke, S.O., Legesse, W., Meester, L and Goethals, P.L.M. (2013). Physico-chemical and biological characterization of anopheline mosquito larval habitats (Diptera: Culicidae): implications for malaria control. *Parasites & Vectors* 2013, 6:320 <http://www.parasitesandvectors.com/content/6/1/320>
- Nikookar, S.H., Fazeli-Dinan, M., Azari- Hamidian, S., Mousavinasab, S.N., Aarabi, M., Ziapour, S.P. (2017) Correlation between mosquito larval density and their habitat physicochemical characteristics in Mazandaran Province, northern Iran. *PLoS Negl Trop Dis* 11(8): e0005835. <https://doi.org/10.1371/journal.pntd.0005835>
- Niyang, E. A, Bassene, H, Fenollar, F and Mediannikov, O. (2018). Biological Control of Mosquito-Borne Diseases: The Potential of *Wolbachia*-Based Interventions in an IVM Framework. *Hindawi Journal of Tropical Medicine* Volume 2018, Article ID 1470459, pp 15 <https://doi.org/10.1155/2018/1470459>.
- Njabo, K.Y., Smith, T.B., and Yohannes, E. (2013). Feeding habits of culicine mosquitoes in the Cameroon lowland forests based on stable isotopes and blood meal analyses. *J Para Vec Bio*. 2013; 5(1):6-12.
- Nkya, T. E., Akhouayri, I., Poupardin, R., Batengana, B., Mosha, F., Magesa, S., Kisinza, W., and David, J. (2014). Insecticide resistance mechanisms associated with different environments in the malaria vector *Anopheles gambiae*: a case study in Tanzania. *Malaria Journal*; 13:28 <http://www.malariajournal.com/content/13/1/28>
- Noori, N., Lockaby, B.G., and Kalin, L. (2015). Larval development of *Culex quinquefasciatus* in water with low to moderate. *J Vector Ecol*. 2015; 40: 208±220. <https://doi.org/10.1111/jvec.12156> PMID: 26611953

- Noutchamae, and Anumudu, C. I. (2009). Entomological indices of *Anopheles gambiae* *Sensu lato* at a rural community in south west Nigeria. *J Vec Bor Dis.*; 46:43-51.
- Okorosobo, T., Okorosobo, F., Mwabu, G., Orem, J.N., Kirigia, J.M. (2011). Economic burden of malaria in six countries of Africa. *European Journal of Business Management* 3:42-62.
- Ononamadu, C.J, Datit, J.T. and Imam, A.A. (2020). Insecticide Resistance Profile of *Anopheles gambiae* Mosquitoes: A Study of a Residential and Industrial Breeding Sites in Kano Metropolis, Nigeria. *Environmental Health Insights*, 14: 1-9 sagepub.com/journals-permissions. DOI: 10.1177/1178630219897272
- Robert, V., Le Goff, G. and Arieu, F.A. (2002). Possible alternative method for collecting Mosquito larvae in rice fields. *Malar J.* 2002;1:4.
- Safiyanu, M., Alhassan, A.J. and Abubakar, A.B. (2016). Detoxification enzymes activities in Deltamethrin and Bendiocarb resistant and susceptible malarial vectors (*Anopheles gambiae*) breeding in Bichi Agricultural and Residential sites, Kano State, Nigeria. *Bayero Journal of Pure and Applied Sciences*, 9(1): 142 – 149. <http://dx.doi.org/10.4314/bajopas.v9i1.22> 142. ISSN 2006 – 6996
- Safiyanu, M., Alhassan, A. J., Imam, A. A., and Abdullahi, H. (2017). Pyrethroids Resistance and Detoxifying Enzymes Activities of Malaria Vector (*Anopheles gambiae*) Breeding in Auyo Irrigation and Residential Sites, Jigawa State, Nigeria. *Annual Research & Review in Biology*; 17(2): 1-8, 2017; Article no.ARRB.36025 ISSN: 2347-565X, NLM ID: 101632869
- Safiyanu, M., Alhassan, A.J., Yayo, A.M., Ibrahim, S.S., Imam, A.A. and Abdullahi. H. (2019). Detection of KDR I1014f mutation in pyrethroids susceptible *Anopheles gambiae* S.L from Ladanai, Kano state, northwest Nigeria. *International Journal of Mosquito Research*; 6(3): 10-15.
- Soko, W., Chimbari, M. J. and Mukaratirwa, S. (2015). Insecticide Resistance in Malaria-transmitting mosquitoes in Zambia: A review. *Infectious Diseases of poverty*, 4: 46. DOI 10.1186/s40249-015-0076-7.
- Trivedi, R.K. and Goel, P.K. (1984). Chemical and Biological Methods for Water Pollution Studies. Karad: Environment Publications. Pg 56
- WHO (2013). World Malaria Report. 2013. World Health Organization, Geneva, Switzerland. (<http://www.who.int/malaria/publications>). Accessed: January, 2014.
- WHO. (2019). World malaria report 2019. Geneva: World Health Organization; 2019. Licence:CC BY- NC-SA 3.0 IGO. ISBN 978-92-4-156572-1
- WHO (2021). World Health Organization, World Malaria Report (2021). <https://www.who.int/news-room/fact-sheet/detail/malaria>. Accessed 28 April 2021
- Wilke, A. B. B. and Marrelli, M. T. (2015). "Paratransgenesis: A promising new strategy for mosquito vector control," *Parasites & Vectors*, vol. 8, no. 1, 2015.