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Morphological identification and Insecticide susceptibility status of Anopheles gambiae s.l mosquitoes in Akoko South West local government area of Ondo State

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Abstract

Lacks of information on susceptibility status, incorrect identification are the major problem facing malaria vector control program in Akoko Southwest Local government area of Ondo State, this study tend to morphologically identify Anopheles gambiae s.l, the major afro-tropical malaria vector and their insecticide susceptibility status, larvae were sampled from pools, stagnant water and tyre using WHO standard dipping method and was transported to the Department of Animal and Environmental Biology department Adekunle Ajasin University, Akungba Akoko where they were reared to adult and insecticide susceptibility test were performed on the reared adult using the CDC bottle bioassay and all mosquitoes assayed were identified morphologically using simple digital light microscope.

The susceptibility test shows a high degree of resistance to both permethrin and deltametrin derivatives. Result from the study revealed that 69 (61%) population were resistant to Deltametrin while 72(64%) were resistant to permethrin, it was discovered that 43(38%) were susceptible to deltametrin, while 40(36%) were susceptible to permethrin. Morphological identification result revealed varied number of Anopheles gambiae s.l from the four sampled location, with Oka-Akoko having the highest sample size 82 (37%) followed with Akungba Akoko 70(31%) Supare 32(14%) and Oba-Akoko 40(18%) with the least population.

Anopheles gambiae s.l was strongly resistant to deltametrin and permethrin insecticides derivatives this may be accrued to inappropriate use and over-exposure of the insecticide to the insect, the presence of Anopheles gambiae complex which are strongly anthropohilic suggest that there will be high burden of malaria incidence in Akoko-south west local government area of Ondo state.

Abbreviations
CDC – Centers for Disease Control and Prevention
WHO – World Health Organization
Anopheles gambiae s.l. – Anopheles gambiae sensu lato

Keywords: Anopheles gambiae s.l., malaria vector, insecticide resistance, deltamethrin, permethrin, morphological identification, CDC bottle bioassay, Ondo State, Nigeria

INTRODUCTION

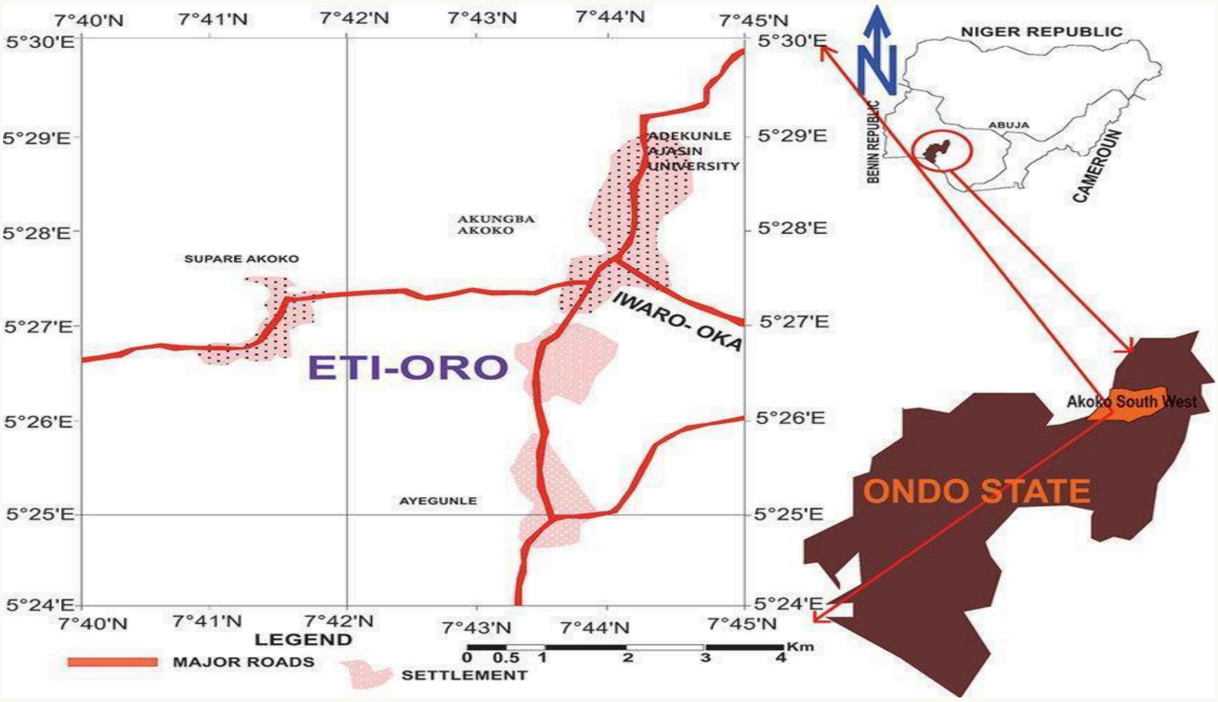
Mosquitoes borne diseases remain a serious public health issue in Africa; transmitting arrays of disease parasites and pathogens ranging from nematodes, protozoa and viruses, (Boccolini, *et al.,* 2012). They are the most serious and complex public health disease in tropical and sub-tropical parts of the world. (Haileselassie *et al.,* 2022). They breed in wide varieties of sites which include stagnant water, fresh water and sewage water; the three predominant species of mosquitoes are *Anopheles mosquitoes*, *Aedes mosquitoes* and *Culex mosquitoes. Anopheles* Mosquito's vector plasmodium which causes malaria in humans and is a serious public health disease of great concern in tropical and sub-tropical parts of the world. Spread of mosquitoes borne disease is a huge public health issue with high proliferations rate in Africa (WHO 2021). Malaria is the most common disease in Africa and about 219 million people are currently affected and 435,000 mortality rates were recorded in 2017 (WHO, 2018). It poses a great setback and critical challenge to public health, causing millions of deaths, particularly infant and pregnant women in Africa (WHO, 2022). Nigeria has the unenviable record of contributing about 25% to world malaria burden (WHO, 2012). *An. gambiae* senso-lato complex is the most capable Afro-tropical vector of malaria this species complex comprises of six morphological undistinguishable species: *Anopheles gambiae* s.s. Giles, *Anopheles arabiensis* Patton, *Anopheles bwambae* White, *Anopheles melas Theobald, Anopheles merus* Donitz and *Anopheles quadriannulatus* Theobald. (Davidson, 1956, 1964, Davidson and Hunt, 1973). These members of the species complex contribute to the high risk of malaria in Nigeria. (Oduola *et al, 2013*). Malaria vector control and elimination programmes depend mainly on two vector intervention tools, indoor residual spraying (IRS) and long-lasting insecticidal nets (LLINs) (WHO, 2016). Most insecticide vector control relies on the use of pyrethroid derivatives (Alpha-cypermethrin, Bifenthrin, Cyfuthrin, Permethrin, Deltametrin, and Lambda-cyhalothrin), (Nkya *et al., and 2020*). Pyrethroid insecticide is not only limited to vector control in public health but also used for Agricultural pest control, although, the success achieved are threatened by several challenges which ranges from, incorrect identification, insecticide resistance and distribution (Awolola, *et al*., 2002, 2005; Ranson, *et al.,* 2011; Ibrahim, *et al.,* 2014; Okorie, *et al*., 2015), The surge and widespread problem of insecticide resistance happened to be one of the major hindrances facing malaria vector control programs in sub-Saharan Africa. This research work provided in-depth knowledge on *An. gambiae* complex identification and their insecticide resistance status in Akoko Southwest Local government area of Ondo State.

METHODS

STUDY SITE

This study cites Akoko Southwest Local Government is located in Ondo State, western region area of Nigeria, one of the country's 36 states, having its capital at Akure. The state nicknamed ''Sunshine State'' is right within the expansive sharing common borders with the State like, Kogi to the Northeast, Delta to the Southeast, Lagos to the South, Ogun to the Southwest, Ekiti to the North, and Osun to the Northwest, Ondo State include mangrove-swamp forest near the Bight of Benin, Edo state. The State has an area of

15,500km^2. The study site Akoko southwest local government is 56km away from Akure the State capital. Akokosouthwest is situated between latitude 7*28 and 5*0E, it shares a boundary with Ikare-Akoko in the North while to the East and West Supare-Akoko, Iwaro-Akoko and Etioro-Akoko to the south, the climate of the sampled sites have two peaks of rainfall. The first peak falls between April and July, while the second peak is between late August and October. These two periods are marked with heavy rainfall



Collection of Mosquitoes

Anopheles mosquito Larval was sampled from their natural ovipositing sites (pool, gutters, potholes) using WHO standard dipping method and were reared to adult at insectary of Animal and Environmental Biology Department, Adekunle Ajasin University, Akungba Akoko. Insecticide susceptibility tests were carried out using Center for Disease Control (CDC) bottle bioassay, both susceptible and resistant populations were morphologically identified with digital portable microscopes.

Evaluation of Susceptibility or Resistance

Insecticides Tested

- Deltamethrin (0.05%)
- Permethrin (0.75%)

Test Procedure

1. **Mosquito Preparation**: Three to five-day-old, non-blood-fed female An. gambiae s.l. mosquitoes were used for the tests.
2. **Exposure**: For each insecticide, four replicates of 20-25 mosquitoes were exposed to insecticide-impregnated papers in a CDC bottle for 60 minutes. Concurrent controls were run using silicone oil-treated papers.
3. **Recovery Phase**: After exposure, mosquitoes were transferred to holding tubes, provided with 10% sugar solution, and maintained at 27±2°C and 70±10% relative humidity.
4. **Mortality Assessment**: Knock-down was recorded during exposure at 60 minutes. Final mortality

was recorded 24 hours post-exposure.

5. **Interpretation of Results**: According to WHO criteria, mosquito populations were classified as:
   - Susceptible: mortality ≥98%
   - Potentially resistant: mortality 90-97%
   - Resistant: mortality <90%

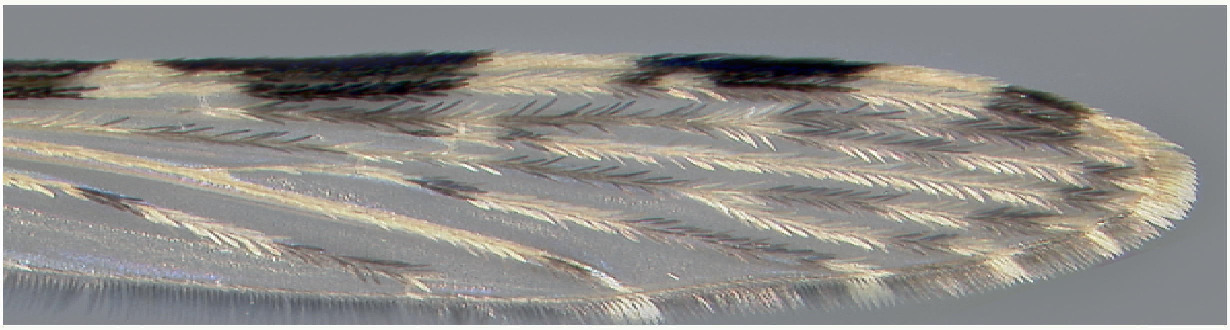
Abbott's formula was applied to correct mortality rates when control mortality was between 5% and 20%. Tests were repeated if control mortality exceeded 20%.

Insecticide Susceptibility Data Analysis

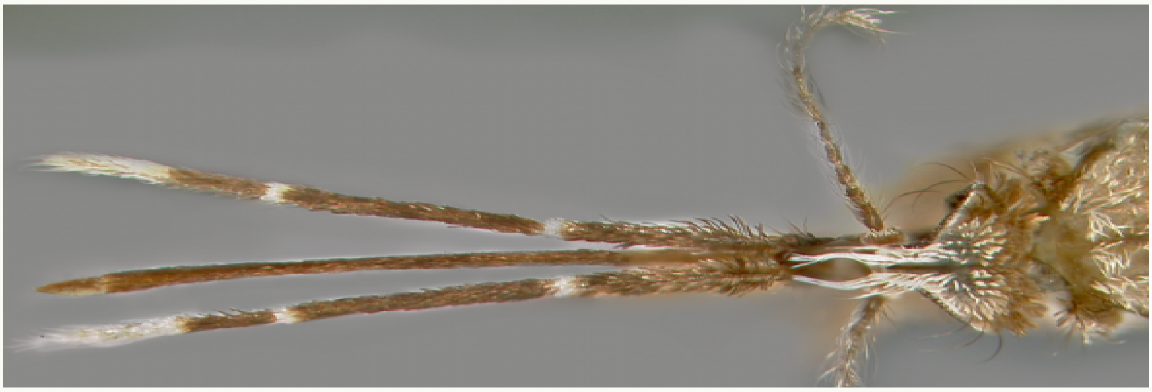
1. **Mortality Calculation**: Percentage mortality was calculated for each insecticide by dividing the number of dead mosquitoes by the total number tested and multiplying by

Morphological Identification

Morphological Identification of *Anopheles gambiae complex* was performed following morphological characters keys of Gilles and Coetzee (2023) under x20 digital light microscope. The keys focused on third apical interrupted dark bound at the upper margins of the wings which are present in *An. gambiae complex*. The palps elongated with the size of proboscis. Palpus usually with 3 pale bands Speckles on the legs, third preapical dark area on vein 1 with a pale interruption and tersi 1-4 with conspicuous pale bands.



***Anopheles gambiae complex* wings**



***Anopheles gambiae* head with accessory**

Data Analysis

Morphological identification result was analysed using simple descriptive analysis same with insecticide susceptibility assay using pie chart, Bar chart, and percentage.

Results

Total mosquitoes sampled at the studied site

	Oka-Akoko	Akungba	Supare	Oba-Akoko	Total
Mosquitoes larva	800	1118	974	520	3412
emerge mosquitoes	547	876	634	401	2458
Morph. Identified	547	876	634	401	2458
An. Gambiae	82	70	32	40	224

3412 mosquitoes larva collected from the four sampled sites of Akoko-south West Local Government area of Ondo State, 2458 (72%) emerged an adult and all emerged mosquitoes were subjected to morphological identification using portable simply light digital microscope 224 (9.1%) are member of *Anopheles gambiae* complex while 2234 (91.8%) belong to Culex and Aedes mosquitoes, *Anopheles rufipes* and *Anopheles coustanii* Culex was the most abundance followed with Aedes and very few *Anopheles rufipes* and *Anopheles coustanii*.

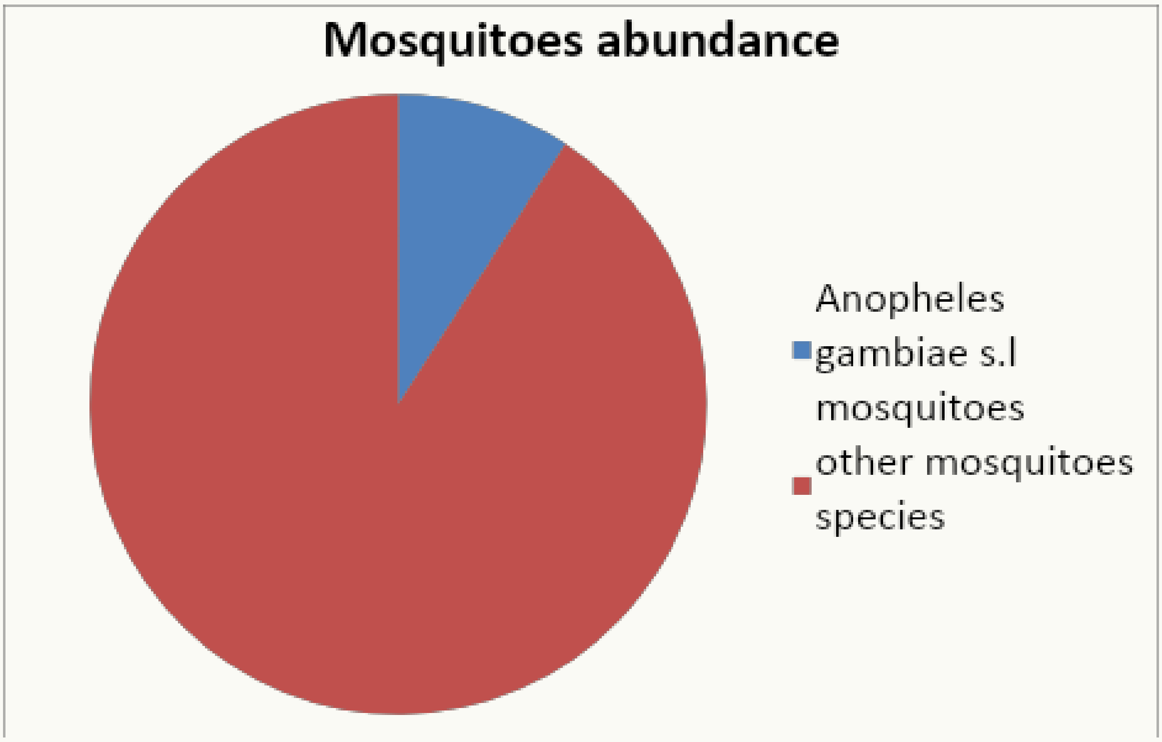


Figure 1: Mosquito's distributions Akoko Southwest Local Government area of Ondo State

Few members of *Anopheles gambiae complex* were found in the sampled locations compared to Culex and Aedes, this may be accrued to water from sampled spots which are polluted and many of the *Anopheles larva* may not be able survive a polluted water. High environmental pollution due to lack of proper drainage, indiscriminate waste disposal, rocky and hilly topography might likely account for the few number of *Anopheles gambiae* complexes recorded in the sampled sites. Though the malaria incidence rates in the studied sites remain high according to information from health facilities this suggests that

Anopheles might be breeding in the closest (breeding inside the toilet water closest and their water reservoir).

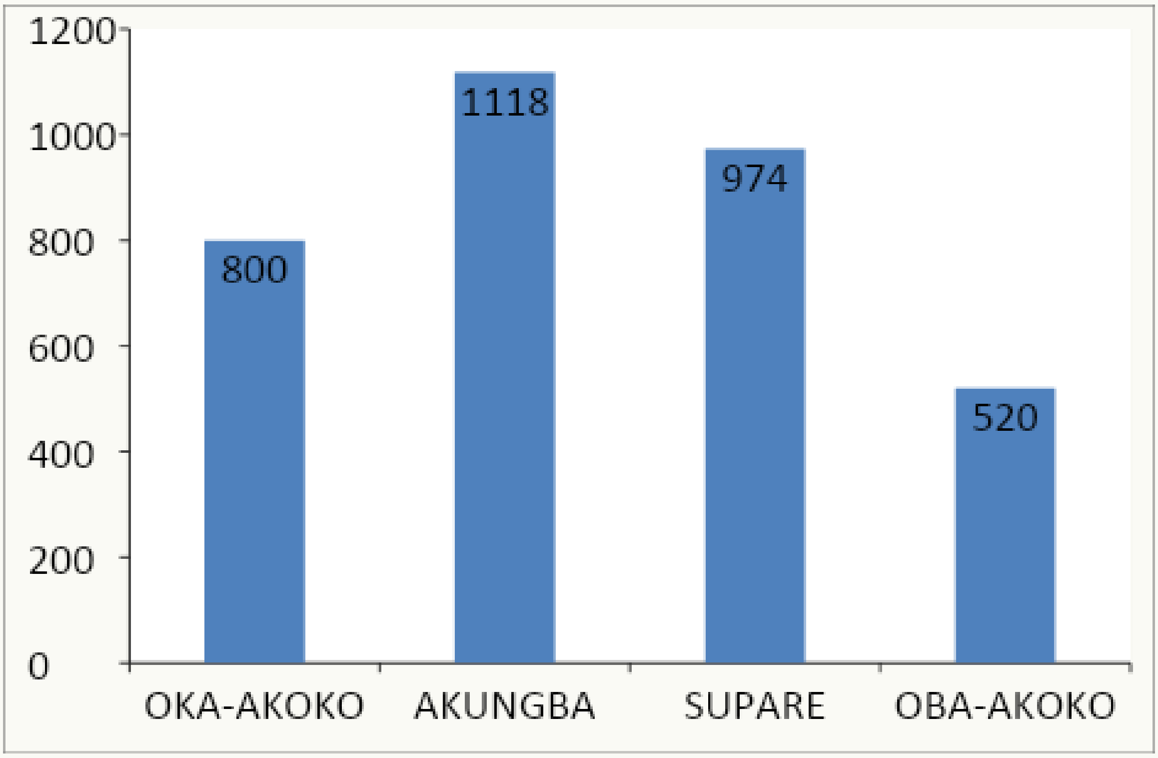


Figure 2: Mosquitoes larva collected

The figure above represents the number of mosquitoes larva collected from the four sampled sites the result varies in different location with Akungba having the highest larva collection followed with Supare, Oka-Akoko and Oba-Akoko, though the highest *Anopheles gambiae* larva collection was recorded in Oka-Akoko, followed with Akungba, Oba-Akoko and Supare.

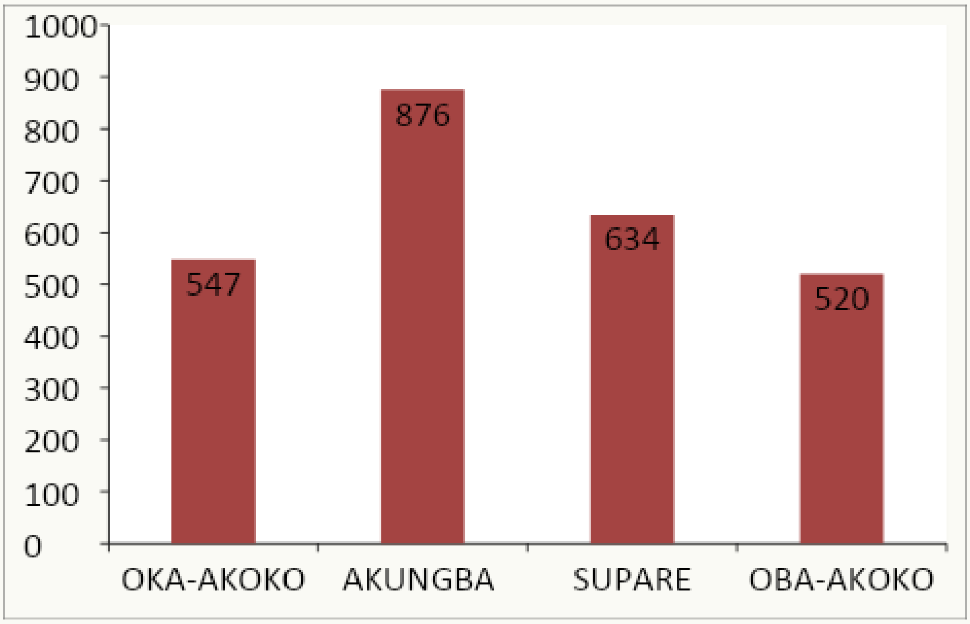


Figure 3: Total emerged mosquitoes

The above figure show the result of the total emerged mosquitoes with Akungba have the highest number of emerged mosquitoes followed with Supare, Oka-Akoko and Oba-Akoko, though this number did not translate to the number of Anopheles gambiae complex that emerged in the study site.

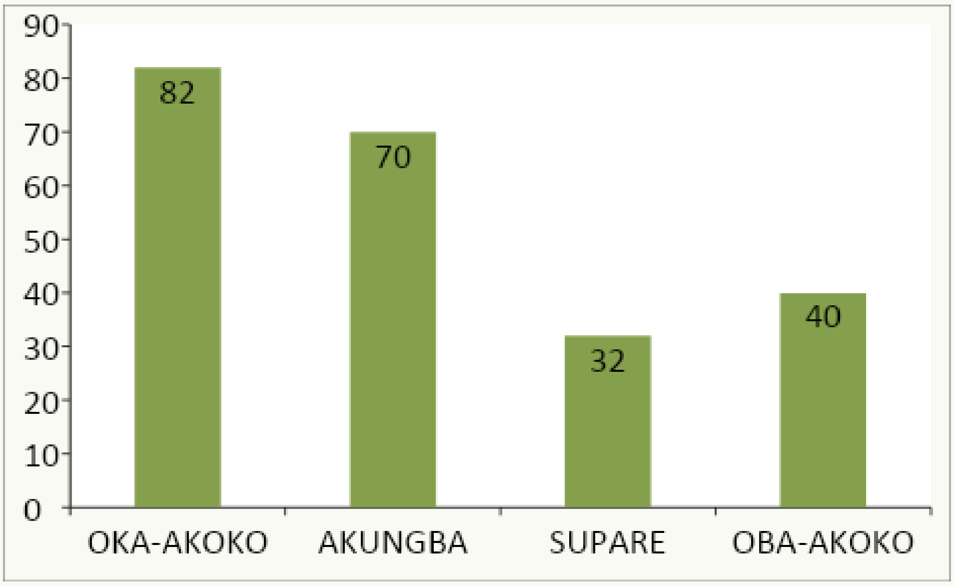


Figure 4: Total *Anopheles gambiae* identified

The number of *Anopheles gambiae complex* collected from the four sampled site varies with Oka-Akoko of having the highest sample size 82 (37%) followed with Akungba Akoko 70(31%) Supare 32(14%) and Oba-Akoko 40(18%) with the least population. This variation may be due to availability of sample sites, the terrain of the sample site and the human population.

Table 1: Insecticide susceptibility result

Insecticide exposed	Deltametrin	Permethrin
Insecticide Conc. used	0.25%	0.25%
Duration of exposure	1hr	1hr
Total exposed *An.gambiae*	112	112
Resistant	42	52
Mortality	22	28
Tolerant	48	32
Active after fed for 24hr	27	2 0
Dead after fed for 24hr	21	12

Table 2: Insecticide Susceptibility Assay

Deltametrin	Permethrin
Total Resistant 69	Total Resistant 70
Total Susceptible 43	Total Susceptible 40

Calculations

$$\frac{deadmosquitoe\ 69\times100}{total\ mosquitoes\ 112} = resistant\ 61\%\ for\ deltamethrin$$

$$\frac{deadmosquitoe\ 72\times100}{total\ mosquitoes\ 112} = resistant\ 64\%\ for\ permethrin$$

$$\frac{deadmosquitoe\ 43\times100}{total\ mosquitoes\ 112} = susceptible\ 38\%\ for\ deltamethrin$$

$$\frac{deadmosquitoe\ 40\times100}{total\ mosquitoes\ 112} = susceptible\ 36\%\ for\ permethrin$$

A total of 224 *Anopheles* mosquitoes were subjected to insecticide susceptibility bioassay for a diagnostic period of an hour following CDC standard protocol, 112 mosquitoes were exposed to deltametrin same with permethrin. The results revealed that 69 (61%) population were resistant to Deltametrin while 72(64%) were resistant to permethrin, it was discovered that 43(38%) were susceptible to deltametrin, while 40(36%) were susceptible to permethrin. None of the two insecticides were able to achieve 95% knockdown at an hour diagnostic time, which failed the cut-off value recommended by WHO (WHO, 2023) for susceptibility. The high resistance discovered in this research work is due to over-reliance on insecticides by households and farmers in the study locations. Each household and farmers possess an insecticide to which other means of vector control are neglected.

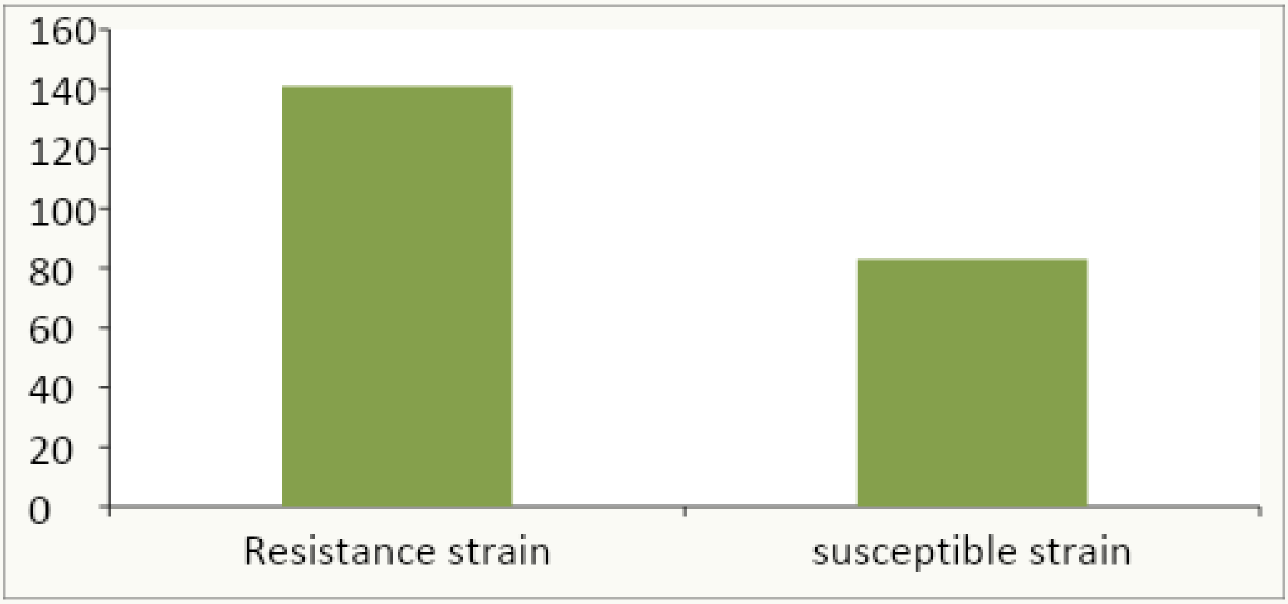


Figure 4: Resistance to susceptibility status

The figure above shows the resistance and susceptibility status of *Anopheles gambiae* complex to both deltametrin and permethrin, 62% were resistant to both insecticides while 37% were susceptible to insecticide.

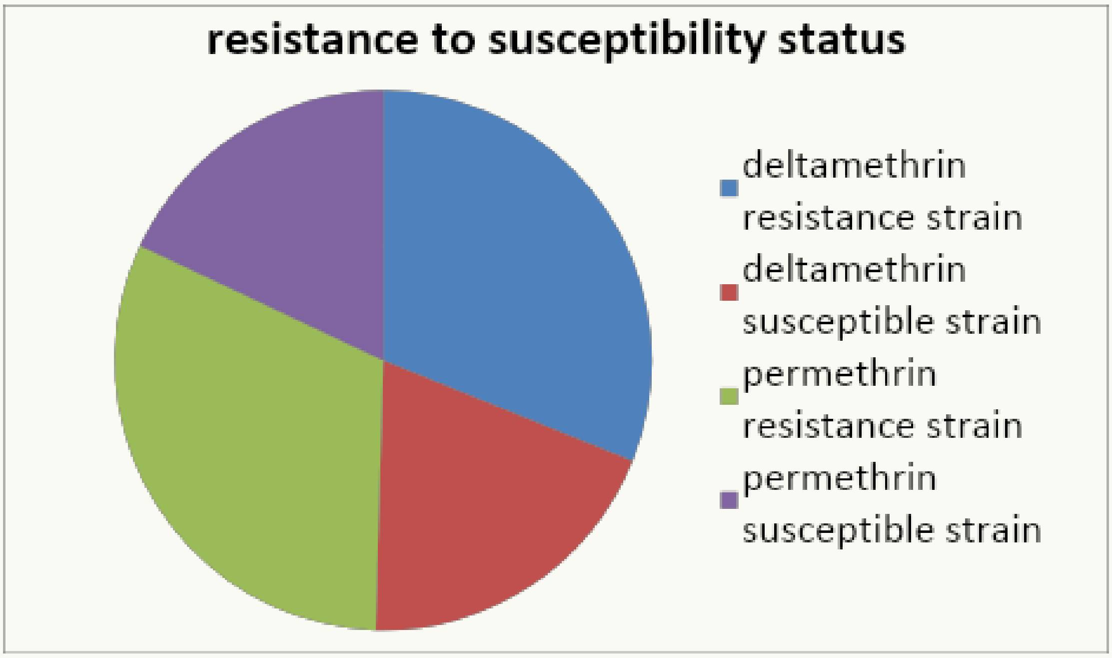


Figure 5: Resistance to susceptibility strain

The above figure show the degree of resistance and susceptibility of the two exposed insecticide with deltametrin derivatives having 42 (37%), lower compared to permethrin with 52(46%) resistant, it was discovered that 22(20%) were susceptible to deltametrin, while 28(25%) were susceptible to permethrin and 48(43%) were tolerant to deltametrin while 32(29%) were tolerant to permethrin,

DISCUSSION

The result from the susceptibility assay shows high degree of resistance to both permethrin and deltametrin derivatives, it revealed that 69 (61%) population were resistant to Deltametrin while 43(38%) were susceptible and 72(64%) were resistant to permethrin, while 40(36%) were susceptible to permethrin, Our findings was in line with the research study on resistance carried out on population of *An. gambiae* in Bauchi, Nigeria which reported resistance to permethrin insecticide (Umar, *et al.,* 2014) and also similar to a studied which reported *Anopheles* mosquitoes resistant to deltametrin (in Ture community; 70% mortality) (Aworetan *et al*.,2022),also agrees with the previous studies from Benin where mortality rate to deltametrin was reported to range from 27%-50% (Aikpon, *et al.,* 2013), Pitoa Cameroon (Etang, *et al.,* 2016). This finding also contradicts reports from Kisumu, Cameroon (Etang, *et al.,* 2016), Ibadan, Nigeria (Ibrahim, *et al.,* 2013) and Yola, Sudan savannah in Nigeria (Balarabe, *et al.,* 2015) where full susceptibility to deltametrin were reported.

The result from morphological identification shows that the member of *Anopheles gambiae complex* are the predominant *Anopheles* species though the number varies with location with Oka-Akoko having the highest sample size 82 (37%) followed with Akungba Akoko 70(31%) Supare 32(14%) and Oba-Akoko 40(18%) with the least population, these findings was in-line with earlier studies, which reported predominantly pure collections of *An. gambiae* complex in Southwestern, Nigeria (Awolola, *et al.,* 2002; Onyabe, *et al.,* 2003).It is also similar to the findings of (Oduola, *et al.,* (2013), where higher abundance *of An.gambiae* was reported with only one *An. arabiensis* in three Local Government areas of Osun state, (Oduola, *et al.,* 2013). It is also in consonance with previous findings which revealed widespread of *An.*

gambiae complex (Okwa, *et al.,* 2009).Similar results have been reported in savannah forest ecological zone in Nigeria (Balarabe, *et al.,*2015).

CONCLUSION

This study revealed the prevalence and insecticide susceptibility status of *Anopheles gambiae complex* in Akoko Southwest local government area of ondo state. The result shows that *Anopheles gambiae* complex cannot strive well in polluted water compared to other species of mosquitoes and there is strong *Anopheles gambiae* resistance to deltametrin and permethrin insecticides derivatives.

Declarations

Ethical Approval and Consent to Participate

- Not applicable

Consent for publication

- All authors give their consent to publish this article

Availability of data and material

- All data and material used in this study are generated by the authors and are available.

Competing interests

- The authors declare that they have no competing interests.

Funding

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Authors' contributions

- I M Aworetan, and V I Olotu, carried out the sample collections and insecticide susceptibility assay of the study, morphological identification of the assayed mosquitoes was done by K D Ileke, B W Adu, and O AFOLABI All authors contributed to the drafting and final writing of the article.

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