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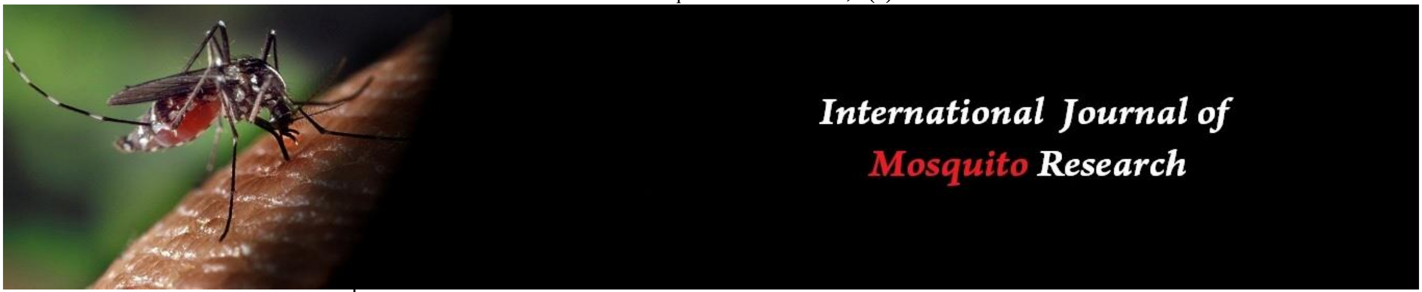

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Public health implication and impact of climatic variables on the mosquitoes breeding in discarded tyres in an urban city of Nigeria

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Abstract
The purpose of the study is to determine the influence of climatic variables on the composition of mosquito species breeding in disposed automobile tyre dumpsite in Mile One, Port Harcourt, River State. A descriptive cross-sectional design was adopted to evaluate vector dominance, distribution, species diversity and seasonal variation on the abundance of mosquitoes at the dumpsite. For larval sampling, hundred dips were randomly collected from 100 sampling tyres twice every week for seven months. Climatic data such as rainfall, temperature and humidity were collected from Nigerian Meteorological Agency, Port Harcourt Station. Inferential statistics such as diversity index, correlation, and regression analysis were carried out at 5% level of significance. Monthly collection revealed that June had the highest collection of larvae 1041 (28.55%), followed by July, 966 (26.49%) while 2532 adult mosquitoes emerged of which *Ae. albopictus* was the highest. Species dominance and richness was highest in males while females recorded the highest in terms of evenness and diversity (Shannon index). More so, the T-test statistic using the Shannon and Simpson index revealed that diversity is significantly different (p-value <0.05) for both sexes. The correlation analysis revealed that mosquito abundance is significantly (p<0.05) related to temperature with a negative coefficient value of 0.639 and correlates positively with rainfall with a positive coefficient value of 0.804. *Ae. aegypti aegypti* was identified as the *Aedes aegypti* species using the DNA characterization. The non-existence of noticeable environmental control of illegal dumping of used tyres in urban and residential areas, coupled with lack of sensitization has made discarded tyres an important breeding habitat for mosquitoes of public health importance in Port Harcourt, River State Nigeria.

Keywords: Mosquitoes, tyres, breeding, dengue

Introduction
The persistent and unprecedented increase in population of mosquitoes over the past decades have been an undesirable consequence of many types of prodigious numbers of parasitic and arboviral diseases as mosquitoes exploit almost all types of aquatic habitats for breeding.
Larvae of mosquitoes have been found to thrive in aquatic bodies such as fresh or saltwater marshes, mangrove swamps, rice fields, grassy ditches, the edges of streams and rivers and small, temporary rain pools. Many species prefer habitats with vegetation while some breed in open, sunlit pools. A few species breed in tree holes, tyres or leaf axils of some plants (Kitching, 2011) [16].
Mosquitoes have been incriminated in the sole transmission of important human diseases including malaria, filariasis, Yellow fever, Dengue fever, Chikungunya, West Nile virus etc (Jackman and Olson, 2019) [14]. These diseases have had serious negative impacts on the economic development as well as medical and social wellbeing of people living in the areas of prevalence (Amiruddin *et al*., 2012) [3]. The vectorial capacity of mosquitoes for the diseases they transmit is largely influenced by the intensity of larval production from breeding habitats (Gosselle *et al*., 2017) [11].
Costanzo *et al*. (2015) [6] noted that one of the habitat mosquitoes used as breeding sites are disposed tyres found in peri-domestic areas which has greatly attracted attention, as their locations close to homes have been reported to have significant effect on human health.

For any vector control measures to be successful, good knowledge of the breeding ecology of mosquitoes including, the types and preferences for larval habitats, spatial and temporal distribution of breeding sites, as well as the physical, biological, and chemical characteristics of the habitats are required (Ferdinard & Kaymart, 2018) [9].

Disposed tyres containing water have been reported to act as breeding sites for mosquitoes (Gonzalez *et al*., 2020; Diniz *et al*., 2017, Adebote *et al*.,2011) [10, 7, 2]. In essence, illegal dumping of tires in urban and wooded areas, coupled with declines in natural mosquito breeding sites due to urbanization has made discarded tires an important source of disease vectors. The purpose of the study is to determine the influence of climatic variables on the seasonal composition of mosquito species breeding in disposed automobile tyre dumpsite in Mile One, Port Harcourt, Rivers State.

Methodology

The study adopted a descriptive cross-sectional design. This design was used because it assesses how frequently, widely, or severely the variables of interest occurring throughout the study area. Also, the design was adopted to evaluate vector dominance, distribution, species diversity and seasonal variation on the abundance of mosquitoes in tyres dumpsite.

Study Area

Port Harcourt City is an urban area where commercial and industrial activities are predominant with lots of industrial waste products such as indiscriminate dumping of tyres. The study work was conducted in a tyre dumpsite- in mile 1, with latitude 4.786307 and longitude 7.003787 (figure 1). Port Harcourt city with an estimated population of 638,360 (NPC, 2011). The study area is characterized by constant rainfall with mangrove/swamp vegetation. The area is highly an industrialized and residential area with vast economic activities.

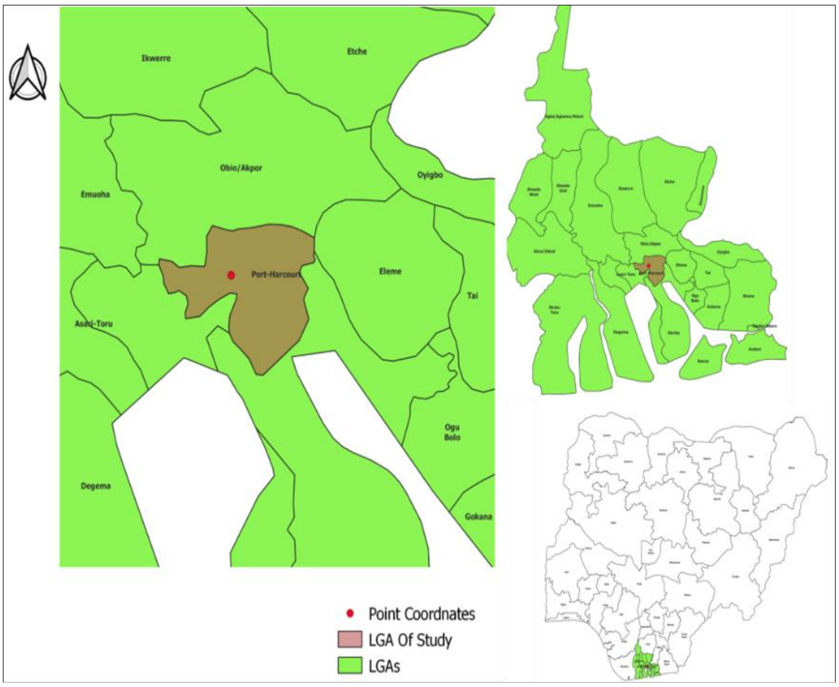

Fig 1: Map of Rivers State showing study site

Mosquito collection and rearing

The larvae and pupae of mosquito were collected with 3mls pipettes from tyres with small water and ladles for tyres with enough water twice every week for a period of seven months from the disposed tyre dump site. With the aid of the ladle, a hundred (100) dips were randomly made in each collection day from 100 marked sampling tyres. All sampling tyres were inspected for the presence of mosquito larva. The disposed tyres with a small quantity of water were sampled with the pipettes. Samples were not collected between February 2020 - April 2020 due to restrictions because of Covid-19 pandemic. The containers containing the mosquito larvae and pupae were carefully carried to the insect laboratory, department of Animal and Environmental Biology, University of Port Harcourt after each sampling for rearing into adult.

Adult mosquitoes that emerged from the larvae were knocked down by aspirating them to a test tube with a dry cotton wool soaked with a very small amount of chloroform. The knocked down mosquitoes were carefully picked with forceps and killed, then dropped on the slide of microscope for identification using the keys of Jupp, (1996) [15] and Hopkin (1952) [13].

Climatic Data

Monthly climatic data such as rainfall, temperature and humidity were gotten from Nigerian Meteorological Agency, Port Harcourt Station. These data were obtained within the duration of nine (9) months of the study but no collection for 2 months due to COVID-19 (May 2019- January 2020).

Statistical Analysis

Data collected was analysed using descriptive statistics such as percentages and charts. Also, inferential statistics such as diversity index, correlation, and regression analysis were carried out at 5% level of significance.

Determining Species Diversity

Species composition was determined using Shannon Wiener Index, species richness was determined with the use of Margalef's Index; while species diversity was determined using Simpson's Index and species evenness was determined with the Evenness Index.

Simpson's index (1949) is given as:

1 – D. where $D = \sum(Pi)^2$, the value of D varies from 0-1 and $Pi = n_i/N$

Where n_i= number of individuals of the ith species, N = total number of individuals for all species.

Shannon – Wiener's (1949) index (H) is given as:

$$H = -\sum[\,Pi\ (\log Pi)]$$

where Pi = (same as in Simpson index).

Margalef's (1958) [18] index of richness

$$d = \frac{S-1}{\log N}$$

where S = total number of species and N = total number of individuals.

Fig 2: The study site

Results

Abundance and distribution of mosquitoes in mile one tyre dumpsite.

Abundance of larvae in the tyre dump sites across the 7 months of study revealed a total number of 3646 mosquito larvae, which was collected from the abandoned vehicle tyres in mile one, Port Harcourt. Monthly distribution revealed that June had the highest collection of larvae 1041 (28.55%), followed by July, 966 (26.49%); August, 645 (17.69%); October, 380 (10.42%); May, 349 (9.57%); November, 221 (6.06%) and September, 44 (1.21%). A total of 2532 adult mosquitoes emerged, with June having the highest number of adults with 783 (30.92%), followed by July 543(21.44), August 457(18.05), October 317(12.52), May 278(10.98), November 119(4.70), and September 35(1.38) respectively.

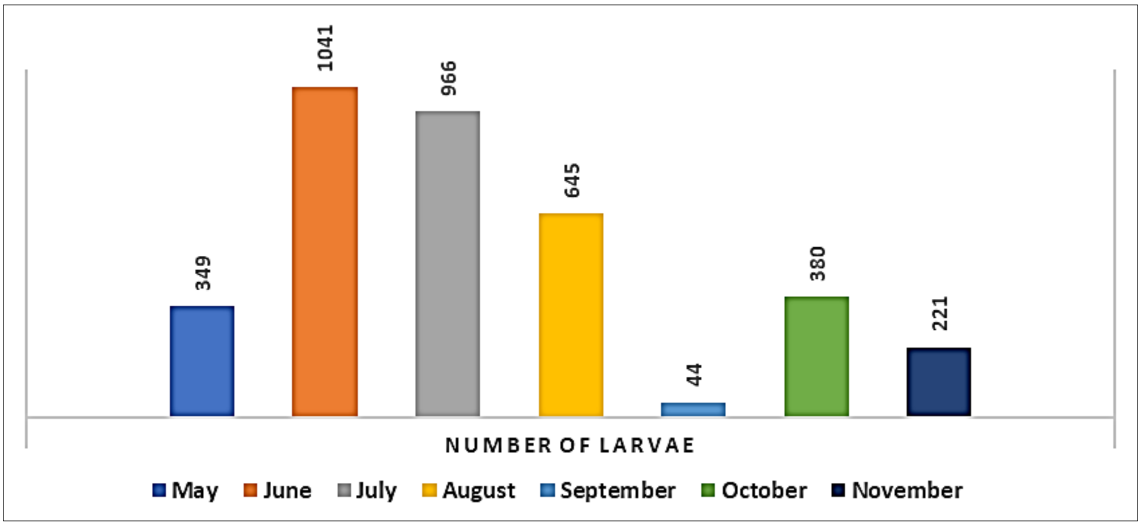

Fig 3: Monthly collection of larvae in disposed tyre dumpsite

Evaluation of monthly abundance and distribution of adult mosquito species in mile one tyre dumpsite.

The distribution of various mosquito species from May to November that emerged from the larvae indicated that in May and June, three species were identified: *Ae.albopictus*, *Ae. aegypti and Cx. quinquefasciatus*. July had four species namely *Ae.albopictus*, *Ae.aegypti*, *Cx. pipiens*, *Cx. quinquefasciatus*; two species in August; *Ae. albopictus* and

Ae. aegypti; four species in September, *Ae. albopictus, Ae. aegypti, Cx. pipiens, Cx. quinquefasciatus*; and four species in November *Ae. albopictus, Ae. aegypti, Cx. quinquefasciatus, Cx. tigripis*. The occurrence of mosquito species in May indicated that *Ae. albopictus* had total number of 228(9.00%), *Ae. aegypti* 40(1.58%) and *Cx. quinquefasciatus* 5(0.20%).

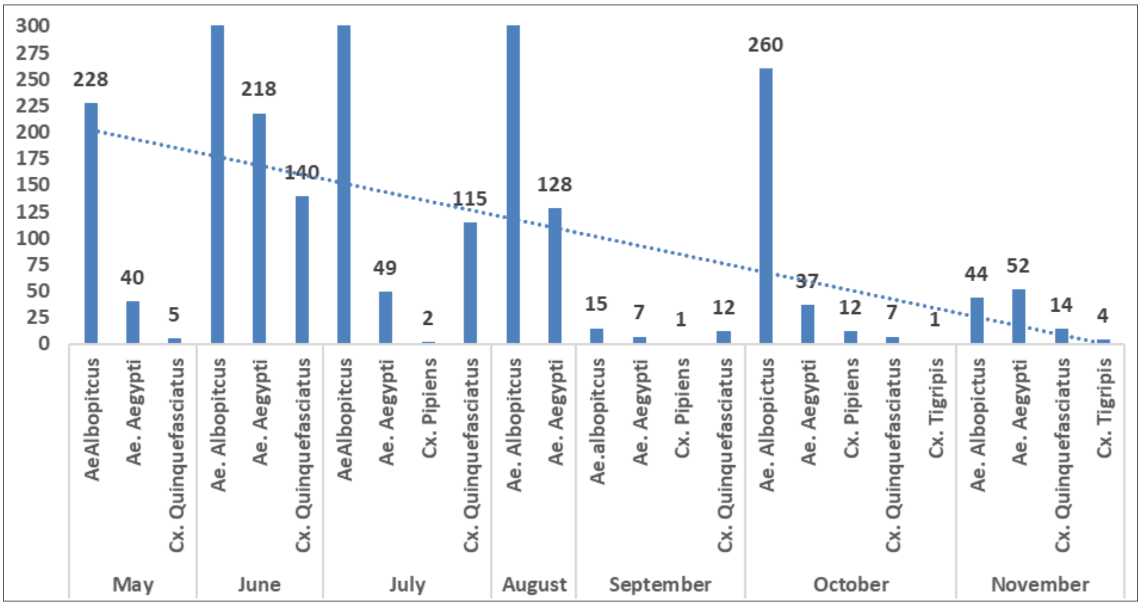

Fig 4: Adult emergence trend across the month

Identification of Mosquito Species Diversity Based on Sex Distribution.

In figure 4, it was shown that of the 2532 total number of adult mosquitoes that emerged in the laboratory, 1715(67.73%) were females while 817 (32.27%) were males. Results also indicated that *Aedes albopictus* was the most abundant female, 1087(63.38%) followed by *Ae. aegypti*, 327(19.07%); *Culex quinquefasciatus*, 286 (16.68%); *Cx. pipiens*, 14(0.82%) while *Cx. tigripis*, 1(0.06%) was least.

Both male and female mosquito appeared in the five species identified. Although, the female mosquitoes collected was higher (1715) than the males (817). The study shows that species dominance and richness was highest in males while females recorded the highest in terms of evenness and diversity (Shannon index). More so, the T-test statistic using the Shannon and Simpson index revealed diversity is significantly different (p-value <0.05) for both sexes.

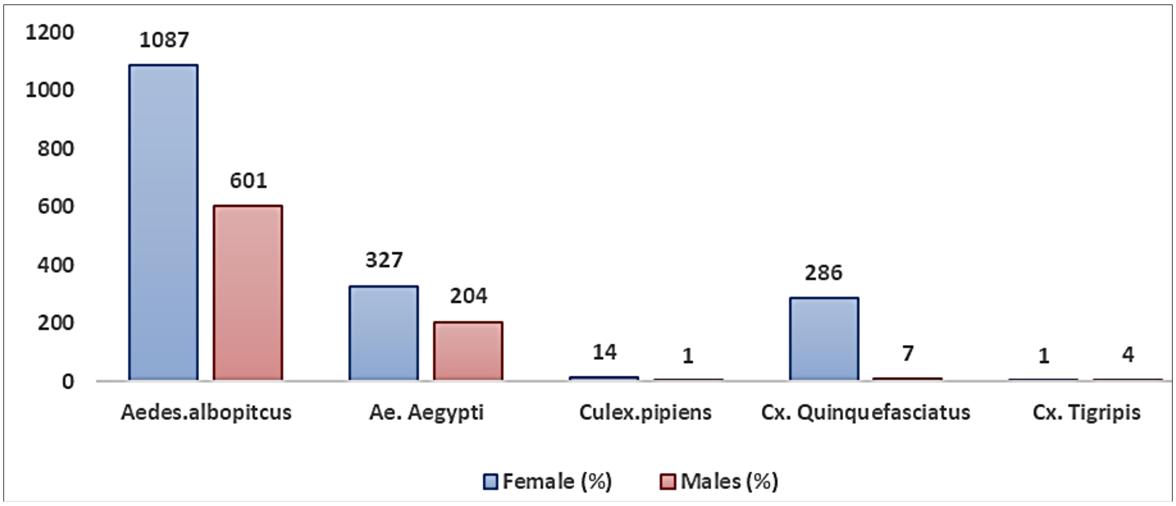

Fig 5: Adult mosquito species based on sex distribution

Evaluation of the influence of rainfall, temperature and humidity on larval abundance.

Table 1 revealed that the highest mosquito abundance was recorded in June followed by July and August with a corresponding rainfall of 2534 mm, 4020 mm and 3036 mm respectively. Also, humidity was 48%, 46% and 52% respectively, while temperature was 25.8 °C, 25.4 °C and 27.3 °C respectively. But September had the lowest adult mosquito abundance (35) with a corresponding 27 °C, 50% and 2200 mm for temperature, humidity and rainfall respectively.

Table 1: Larval abundance and mean temperature, humidity and rainfall based on months.

Months	larval Abundance	Mean Temperature (°C)	Mean Humidity (%)	Mean Rainfall (mm)
May	278	26.7	54	2034
June	783	25.8	48	2534
July	543	25.4	46	4020
August	457	27.3	52	3036
September	35	27	50	2200
October	317	29.3	56	1870
November	119	30.8	56	1350

Relationship between climatic variables and mosquito larval abundance.

The correlation analysis revealed that mosquito abundance is significantly (p<0.05) related to temperature with a negative coefficient value of 0.639 and this implies that increase in temperature resulted to a corresponding decrease in mosquito abundance. However, the regression analysis revealed that temperature is not a significant (p>0.05) predictor of mosquito abundance.

For the humidity (%), correlation analysis revealed that mosquito abundance does not significantly (p>0.05) correlate with the climatic variable with a negative coefficient value of 0.486. Correlation analysis also revealed that species abundance significantly (p<0.05) correlates the amount of rainfall (mm) with a positive coefficient value of 0.804. This increase in rainfall resulted to a corresponding increase in species abundance as the regression analysis revealed that the amount of rainfall significantly (p<0.05) predicts species abundance

Molecular Identification of adult mosquitoes

DNA extraction

The DNA was extracted from the mosquitoes using the kit method at National Arbovirus and Vectors Research Centre, Enugu. The kit was sourced from Zymo Research Company, USA. The manufacturer's instructions were strictly adhered to.

DNA amplification

A commercially prepared master mix was purchased from Inqaba Biotec West Africa. The master mixing components are as follows; commercially prepared One Taq 2X master mix 14µl, Forward primer 2µl, Reverse primer 2µl, DNA template 3µl, the PCR volume was made up to 30µl using Nuclease free water. The thermal cycling condition adopted for the amplification are Initial denaturation at 95ºC for 90sec, for a total of 35 cycles, denaturation @ 95ºC for 30sec, Annealing at 68ºC for 30sec and Extension @72ºC for 1 min. The reaction was held at 72ºC for 5mins for final elongation.

ACE QUIN ------5'-CCTTCTTGAATGGCTGTGGCA-3'

B1246------- 5'-TGGAGCCTCCTCTTCACGGC-3'

Reverse primer for *Aedes albopictus*….. 5'- GTA CTA GGC TCA CTGCCA CTG A-3'

Reverse primer for *Aedes aegypti*……. 5'- TAA CGG ACA CCG TTC TAG GCC CT-3'

Forward primer for both………………18SFHIN 5'-GTA AGC TTC CTT TGT ACA CAC CGC CCT T-3'

Gel electrophoresis

The amplified DNA was run on 2% agarose gel for 1 hour at 120 volts. For visibility under a UV transilluminator the bands were stained with Ethidium bromide. The expected band size for *Culex quinquefasciatus* using this set of primer design is 296bp.While *Aedes albopictus* and *Ae. Aegypti* primer design was 550bp and 515bp respectively. The set of primer sequence used are forward- Reverse primer. This amplification protocol was adopted and modified from already published work by Fanello *et al,* (2002) [25].

Discussion

This study has revealed that there is high abundance of larvae in used tyre dumpsite in the study area as this could be attributed to the environmental conditions which favour the survival of the mosquito larvae in the microhabitats. Dumped tyres containing water have been reported to act as breeding sites for mosquitoes (Gonzalez *et. al*., 2020; Diniz *et. al*., 2017) [10, 7]. In this study, the highest collection of larvae and adult emergence was in the month of June with abundance of 1041(28.55%) and 783(30.92%) respectively. This is usually the onset of the raining season when the eggs are not easily dislodged or dried up due to drought in their breeding sites. However, there was presence of mosquito larvae throughout the period under study due to the continuous rainfall which made water available in the breeding microhabitats.

According to Rubio, (2011) [20], discarded tyres are important for the proliferation of *Aedes* spp. and *Culex* spp. both in domestic and public environments. In this study, five species from two mosquito genera were found. The species found in this study is fewer compared with a similar study of Afolabi *et al*., (2019) [1]. The public health importance of the vectors collected in the study area is that they are potential transmitter of disease outbreak like dengue, yellow fever and filariasis. *Ae.aegypti aegypti* was identified as the *Aedes aegypti* species using the DNA characterization. Kumar *et al*., (2022) [17] stated that only two subspecies of *Ae.aegypti* have been widely recognized namely, *Ae. aegypti formosus* and *Ae. aegypti aegypti* and they vary in their ecology, behavior and morphology. *Ae. aegypti aegypti* as a form is domestic and anthropophilic in nature and this corroborates with the molecular findings as the other form *Ae. aegypti formosus* is a sylvatic that resides primarily in forest areas.

Dengue fever virus are transmitted from to the other by *Aedes* mosquitoes most often *Ae.aegypti* and *Ae.albopictus* (Otuu *et al*., 2019, Bhatt *et al*., 2013) [5, 22]. In 2018, dengue fever virus outbreak was recorded in the University teaching hospital (UPTH) where a 44-year female nurse living in a nearby bushy and swampy environment presented herself with the symptoms. In the case of, Yellow fever outbreaks in Nigeria between 2017 and 2020, Rivers state has been on the map and vector samples (mostly *Aedes* spp.) near the homes of the index cases were collected for viral isolation (WHO, 2019) [23].

The distribution of mosquito species based on months in this study revealed that *Ae. albopictus* and *Ae.aegypti* were observed in all seven months of study, while *Cx. quinquefasciatus* was observed in all months except in August. The species collected in this study had previously been reported in the study of Simon-Oke & Olofintoye (2015) [21], who studied the effect of climatic factors on the distribution and abundance of mosquito vectors. The provision of conducive environment for the breeding of *Aedes* species and *Culex* species in the study area pre-disposes the population of diseases such as chikungunya, lymphatic filariasis, rift valley fever, encephalitis, West nile fever, dengue, and yellow fever (WHO, 2020) [23]. *Aedes* species present in all months of study in the study area plays key role in the transmission of a range of arboviral infections.

Apparently, species dominance and richness were highest in males while females recorded the highest in terms of evenness and diversity (Shannon index). Result based on Shannon and Simpson index revealed both sex diversities was significantly different. The occurrence of *Aedes* mosquitoes breeding in the study area showed that probably it has more preference for breeding in tyres than other habitats. The finding agrees with the study of Gonzalez *et al*., (2020) [10] and Haruna & Abdulhamid (2019) [12] in Minna, Niger State. Female *Aedes* mosquito obtained in all months is within the critical range associated with Dengue virus and yellow fever transmission which an outbreak was confirmed in Rivers State in 2018 with a 100% case fatality rate (WHO 2019) [23]. Therefore, the people living within the area of study are prone to outbreak of arboviral infection once there is the virus in circulation.

The study revealed that species abundance was highest in rainy season with high humidity and low temperature than the dry season as increase in rainfall corresponds to increase in species abundance. Mosquitoes are widely distributed as the nature of the breeding site can be influenced directly or indirectly by various environmental variables such as temperature, humidity, rainfall etc (Mutero *et al*., 2020) [19]. The findings of Eze *et al*., (2018) [8] reported that larval abundance in Port Harcourt city is influenced by rainfall and temperature, as raining season had highest number of larvae and species composition of mosquitoes compared to dry season.

The non-existence of noticeable environmental control of illegal dumping of tyres in urban and residential areas, coupled with lack of sensitization has made discarded tyres an important breeding habitat for mosquitoes of public health importance. Further research on the physio-chemical parameters also needs to be studied to properly infer why Anophelines are absent in that study site despite the uprising of *An.stephensi* in Africa which breeds also in tyres (Balkew *et al*., 2020) [4]. Next study on the mosquitoes breeding on discarded tyres will focus on viral isolation on the mosquitoes collected around the study area.

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