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Research Article

Assessment of Indoor and Outdoor Resting Adult Female Mosquitoes Density Exhibiting Vectorial Portent, Insight from Kano State, Nigeria

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ABSTRACT

Mosquito proliferation has been on the increase in most communities of Kano State, leading to a high rate of mosquito-borne diseases, pregnant women, and infant mortality. Mosquitoes spend the daytime resting in human dwellings, frequent survey of these mosquitoes is needed in vector control for breaking disease transmission. Despite this, data on mosquitos' density are frequently missing. In this study, we have collected and identified all samples of indoor and outdoor resting mosquito species to assess female density. A longitudinal survey was carried out in three different sentinel sites (Darmanawa, Wudil, and Baba Ruga) for the period of 6 months (August 2023 to March 2024). Indoor mosquitoes were collected using the pyrethrum spray Method, while the outdoor mosquitoes were collected using a cardboard box. The knockdown mosquitoes were transported to the laboratory for identification, using a digital LCD microscope to gender and species level with the aid of different taxonomic guides. A total of one thousand and five (1005) female mosquitoes belonging to 5 genera and 21 species were caught. The result showed a significant influence ($p < 0.05$) of species, months, resting behavior, and season on the density of adult female mosquitoes while collection sites do not influence adult female mosquitoes ($p > 0.05$). We concluded that adult female mosquitos' density is affected by species, months of the year, resting behavior, and season, as well as the high density of *An. gambiae* and *Cx. quinnquefasciatus* recorded is a bio-portent of malaria and filariasis spotlight in these communities.

Keywords: Indoor, Outdoor, Resting, Density, Female Mosquitoes

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INTRODUCTION

Mosquito surveillance is fundamental in the control of transmitted diseases, however, a better recognized entomological index of some mosquito-borne diseases is the abundance of adult mosquitoes when compared to egg, larval or pupal indices (Sivagnaname and Gunasekaran, 2012). Studies such as those conducted by Service and Townson (2002) and Harbison *et al*. (2006) showed that, the sampling of adult mosquitoes is required for an entomological surveys associated with malaria prevention and control effort. Adult mosquito surveillance refers to the conception of the population dynamics and species distribution in a given area (Vector Disease Control International, 2021). Mary *et al*. (2020) identified that abundance and species composition of adult mosquitoes indoor or outdoor and subsequently the risk of diseases are influenced by eggs laying behavior in a broad range of aquatic niches. If the abundance and species composition of these mosquitoes are left unchecked, as Li *et al*. (2020) claims, it will further increase the existing global threat of mosquito-borne diseases. An interaction between infectious agents, vector and susceptible population is necessary for a mosquito borne disease to extant and proliferate in a particular environment (Hakizimana *et al*., 2018). Mosquito a Spannish or Portuguese word, meaning little fly. According to Britannica dictionary "Mosquito is a small flying insect that bites the skin of people and animals and suck their blood". A Nematoceran fly with slender small to medium size body (body length 4-3mm), head with noticeable proboscis in male and female which are scaly and forwardly bulging (Kirk-Spriggs and Sinclair, 2017). Seda and Horrall (2023) mentioned that the majority of global insect bites are from mosquitoes which are vectors of rising number of diseases. Therefore, mosquito is a major public health problem and the main cause of vector-borne diseases. Elements that stimulate the position of mosquitoes in transmission of diseases include density, resting and feeding behavior, host preference and adult longevity (Black and Kondratieff, 2005; Sindato *et al*., 2011). A large part of mosquito gonotrophic cycle is spent resting (Paajmans and Thomas, 2011).

Conversely, mosquito resting behavior can be categorized into endophilic mosquito, that rest indoor, inside human shelter, through the period between the end of blood feeding and the beginning of search for an ovipostion site (Pates and Curtis, 2005). The other is exophilic mosquito that spend this period somewhere outside the human habitation (Paajmans and Thomas, 2011). Despite this categorization, resting behavior appear to be somewhat flexible with considerable potential for variation between and within species (Tirados *et al*., 2006). As such the little studies on host seeking mosquitoes focused mainly on a particular female mosquito species. This study aimed to evaluate the density of all female mosquito species resting indoor and outdoor in human dwellings.

MATERIALS AND METHODS

Study Sites

The map of Kano State was used as a sampling frame to select the sampling sites. The sampling sites were selected in two stages (multi-stage sampling); The clusters of 44 Local Government Areas into urban, semi-urban and rural by Kano State Gazette (2015) was adopted as the main sampling level (Figure 1), while a ward within a Local Government Area from a cluster as the primary sampling units (PSUs). Because this reduced the number of sites and cost, to reach the desired accuracy. These include Tarauni (urban) with lager drainage, Wudil (semi-ubarn) with Wudil River and Makoda (rural) with Tomas Dam. From each Local Government Area a ward was randomly selected (Fig. 1) based on proximity to the possible permanent breeding site for the collection of resting adult mosquito species.

Study Design

A quantitative research method was employed in assessing the density of indoor and outdoor resting female mosquitoses in some parts of Kano State. An observational study encompassed descriptive design was used for this study to allow for longitudinal sample collection of resting mosquitoes, once monthly for the period of 6 months (from August 2023 to March 2024).

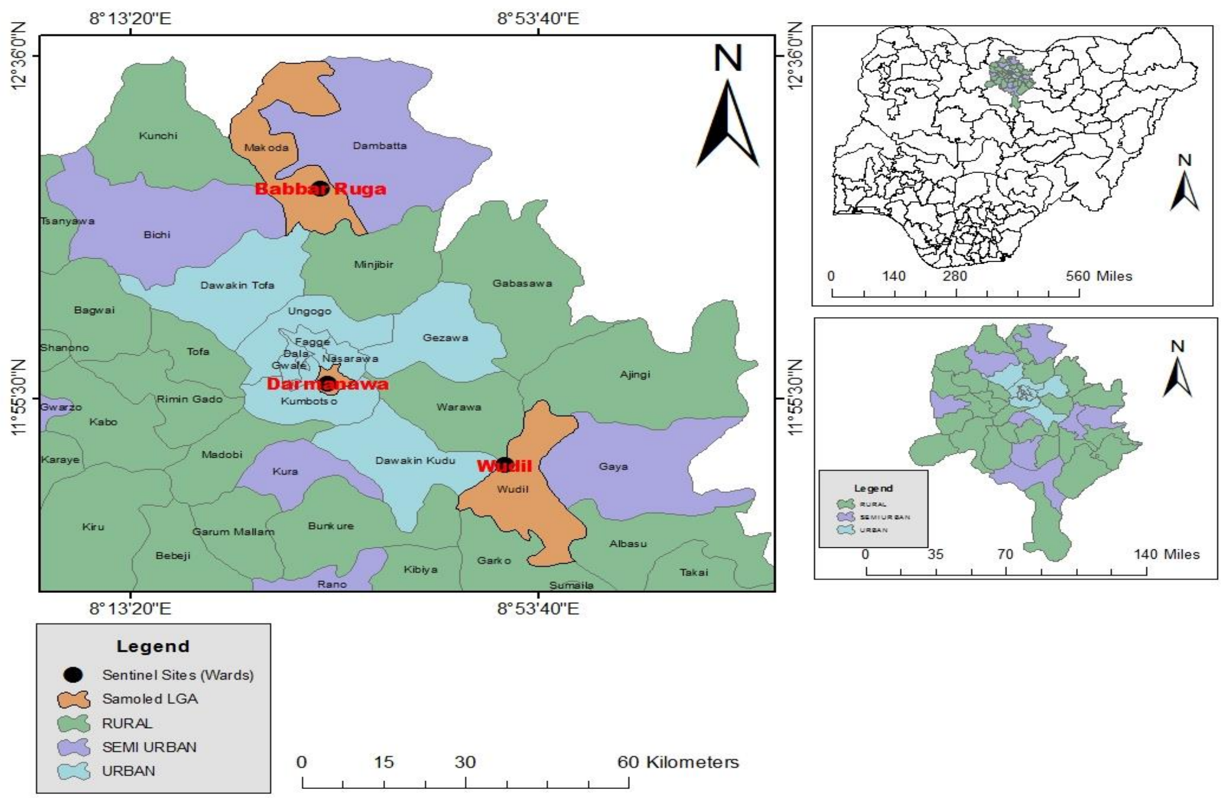

Figure 1: Maps of Study Sites

Indoor Resting Adult Mosquito Collection

Collections of indoor resting adult mosquitoes were done in the morning from 8:00am to 10:00am using pyrethrum spray collections (PSCs) method for the study period, in order to estimate the number of mosquitoes resting inside rooms where people sleep in the night and rest during the daytime. Collections were made using Gongon Triple Action aerosol (Imiprothrin: 0.10%, Transfluthrin: 0.25% and Permethrin: 0.50%; Gongoni Company Ltd.), other materials used include; torch, petri-dishes, white cloth sheet (336cm x 244cm), forceps, labels and containers for carrying samples (Williams and Pinto, 2012). The procedure of WHO (1975) and Harbison *et al*. (2006) was adopted. Households were numbered and ten (10) households were selected at random from each ward for the indoor mosquito collection. In each household only one room was used and the inhabitants were invited to remove food and drinking items from the room, the area of the room was covered wall to wall with the white sheet so that no mosquitoes are lost and the windows are closed. After the floor of the room has been completely lined with the sheet, the collector sprayed the Gongon aerosol. The collector leaved the room and closed the door after the room is filled with the insecticide mist. Ten minutes later the door was opened and mosquitoes were collected with forceps into the labeled petri-dishes containing silica gel and transported to the laboratory for examination.

Outdoor Resting Adult Mosquitoes Collection

Outdoor resting adult mosquitoes were collected according to the procedure of Kweka *et al*. (2009) with slight modification in terms of the cardboard box demission and the positioning of the box. Because this aid in evaluating the species that habitually rest outdoor and their densities. A cardboard box of demission length 35cm by height 37cm by width 24cm, inside lined with black cloth was positioned in an undisturbed area of the household compound, vertically to the surface floor so that the entry is on top side of the box, using a board pin a piece of net was hung by side of entry which was used to covered the entry before the sprayed of Gongon aerosol to prevent escape of the mosquito. The collection made from two households selected randomly was done from 6:00pm to 6:00am and the knock down mosquitoes in the box were collected with forceps into a labeled petri-dishes containing silica gel and transported to the laboratory for examination.

Mosquito Identification

The adult mosquito samples collected, in the laboratory were mounted on *ELIKLIV* digital LCD microscope Model: DM4, to identify the sex morphologically from their palps and proboscis using a guide provided by Service (2012). The mosquitoes were further identified morphologically to species level using reported taxonomic keys by Becker *et al*. (2010), Pecor (n.d.), Coetzee (2020), WHO (2020), and Ramberg (2017).

Indoor and Outdoor Resting Density

The resting density of female adult mosquitoes from the sentinel sites was determined using the formula by Williams and Pinto (2012) below, with slight modification in terms of the number of nights which was not considered, because our collections were made in the daytime. The data for adult female mosquitoes resting indoors and outdoors were evaluated separately. However, this method realized the numerical potency of a mosquito species in a unit area sampled.

$$\text{Density (D)} = \frac{\text{Number of mosquito species}}{\text{Number of houses}}$$

Data Analyses

The morphological characters considered for the identification of female mosquitoes were used to visualize the similarities between species, Neighbor-Joining (NJ) phenetic trees were constructed in PAST 4.03. Using Kimura parameter distances, branch support of the NJ tree was assessed by bootstrapping with 1000 replications, and the calculated values for the density were put through the Shapiro-Wilk test for normality (0.708, $P < 0.05$) to check for the normal distribution of the data in PAST 4.03. The square root transformed data were subjected to one-way analysis of variance (ANOVA), where the ANOVA indicated significant difference, pairwise differences was determined using the Least Significant Difference (LSD) post-hoc test at significance level of 0.05. This analyses was carried out using SPSS 20 statistical software.

Ethical Approval and Informed Consent

Ethical approval for the research with assigned approval number (NHREC/17/03//2018) dated 2nd March, 2022 was obtained from Kano State Ministry of Health research Ethics Committee. The informed consent of the head of a household was sought by filling the designed consent form before the commencement of the mosquito collection.

RESULTS

A total of (n=1005) mosquitoes belonging to 5 genera and 21 species were caught from the sites during the study. Eight hundred and forty-eight (848) were caught indoors while one hundred and fifty-seven (157) were caught outdoors. The tree (Fig. 2) showed the species identified were grouped correctly based on their genera with a few exceptions. *Mansonia uniformis* and *Anopheles annularis* were the most divergent species. Most species from *Anopheles* clustered together with greater levels of similarities. However, *Anopheles nigerrimus* had some morphological similarities with *Aedes geniculatus* and *Aedes venxa*. In the genus *Culex*, species clustered with higher similarities at different levels: *Culex pipiens*, *Culex tritaeniohynchus,* and *Culex torrentimus.* On the other hand, *Culex erythrothorax*, *Culex theileri* and *Culex univittatus*. Morphological similarities were observed between *Culex tarsalis* and *Psorophora ciliata*, and also between *Culex quinnquefasciatus* and *Aedes atropalpus*.

To test the hypothesis that mosquito density is not significantly influenced by species, months of collection, sites, resting behavior, and seasons; rainy and non-rainy periods, we set mosquito density as a dependent variable and species, months of collection, sites, resting behavior, season as independent variables/predictor variables. Mosquito species density was found to be influenced by the species of mosquito identified ($F = 2.406$, $P = 0.002$), with density of *Anopheles gambiae* ($M = 1.589$, $SD = 0.719$) higher on average than the *Culex quinquefasciatus* ($M = 1.275$, $SD = 0.205$) and *Culex pipiens* ($M = 0.958$, $SD = 0.212$). On the other hand, *Aedes atropalpus* ($M = 0.100$, $SD = 0.000$), *Anopheles domicolus* ($M = 0.100$, $SD = 0.000$) and *Anopheles nigerrimus* ($M = 0.100$, $SD = 0.000$) had the lowest density, followed by *Psorophora ciliata* ($M = 0.140$, $SD = 0.013$) (Table 1), this contradicts the hypothesis that density of mosquito is not affected by species and in fact suggests species effect. The density of mosquito species was influenced by months ($F = 3.084$, $P = 0.005$), with November ($M = 1.322$, $SD = 0.403$) having the higher density of mosquitoes, followed by August ($M = 1.240$, $SD = 0.507$) and September ($M = 1.200$, $SD = 0.225$). However, the month of January ($M = 0.393$, $SD = 0.080$) had the lowest density followed by March ($M = 0.494$, $SD = 0.099$) (Table 2), this opposes the hypothesis that density of mosquito is not affected by months and indeed proposes monthly effect. Also density of mosquito species was significantly influenced by resting behavior (F = 18.123, P = 0.000) with higher density of mosquito resting outdoor (M = 1.238, SD = 0.193) as compared with indoor resting mosquitoes (M = 0.582, SD = 0.264) (Table 3), this negates the hypothesis that density of mosquito is not affected by resting behavior and certainly implies resting behavior effect. The density of mosquito species was not influenced by sites of collection (F = 2.811, P = 0.063) with Darmanawa having the marginally higher density (M = 1.000, SD = 0.229), followed by Baba Ruga (M = 0.780, SD = 0.382) and Wudil had the lowest density of mosquito species (M = 0.571, SD = 0.148) (Table 4), this confirms the initial hypothesis that density of mosquito is not affected by sites. Density of mosquito species was significantly influenced by season (F = 2.341, P = 0.003), with rainy season having the higher density of mosquitoes (M = 1.362, SD = 0.423) as compared with the dry season (M = 0.694, SD = 0.221) (Table 5), this also contradicts the hypothesis that density of mosquito is not affected by season and indeed proposes season effect.

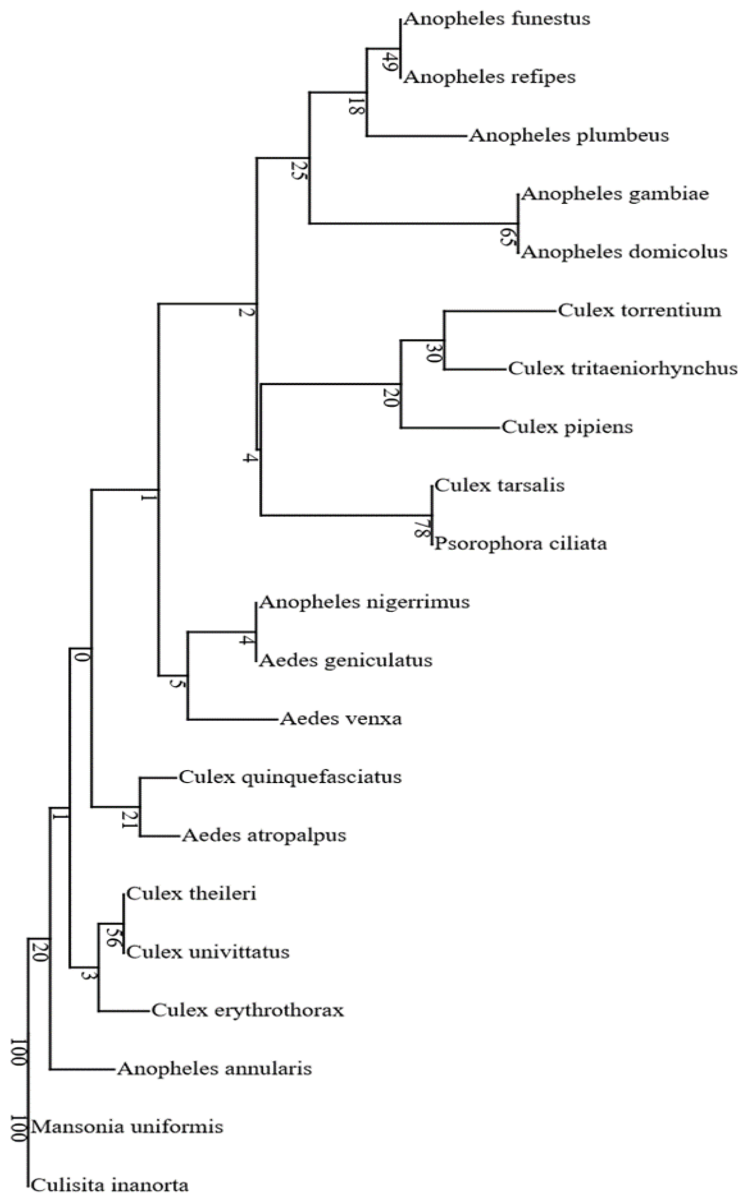

Figure 2. The Neighbor-Joining tree for mosquito species identified derived from the morphological character

Table 1: Mean Density of Adult Mosquito Species Collected

Mosquito Species	Mean Density±SD
Aedes atropalpus	0.100±0.000[a]
Aedes geniculatus	0.333±0.034[ab]
Aedes venxa	0.200±0.000[ab]
Anopheles fenestus	0.200±0.000[ab]
Anopheles annularis	0.594±0.413[ab]
Anopheles barberellus	0.146±0.009[a]
Anopheles domicolus	0.100±0.000[a]
Anopheles gambiae	1.589±0.719[b]
Anopheles nigerrimus	0.100±0.000[a]
Anopheles plumbeus	0.799±0.000[ab]
Culex erythrothorax	0.478±0.102[ab]
Culex pipiens	0.958±0.212[ab]
Culex quinq.	1.275±0.205[ab]
Culex tarsalis	0.262±0.076[ab]
Culex theileri	0.605±0.155[ab]
Culex torrentium	0.446±0.108[ab]
Culex tritaeniorhynchus	0.222±0.018[ab]
Culex univittatus	0.243±0.043[ab]
Culiseta inornata	0.333±0.034[ab]
Mansonia uniformis	0.262±0.076[ab]
Psorophora ciliate	0.140±0.013[a]

Mean±standard deviation with the same letter within the column are no significantly different from each other (Least Significant Difference P<0.05).

Table 2: Mean Density of Adult Mosquito species Collected within the Months of Collection

Month	Mean Density±SD
August	1.240±0.507[bc]
September	1.200±0.225[bc]
October	0.865±0.337[bc]
November	1.322±0.403[b]
December	0.870±0.282[bc]
January	0.393±0.080[a]
February	0.636±0.138[ac]
March	0.494±0.099[ac]

Mean±standard deviation with the same letter within the column are not significantly different from each other (Least Significant Difference P<0.05).

Table 3: Mean Density of Adult Mosquito species Collected within Resting Behaviour

Resting Behaviour	Mean Density±SD
Indoor	0.582±0.264[a]
Outdoor	1.238±0.193[b]

Mean±standard deviation with the same letter within the column are not significantly different from each other (Least Significant Difference P<0.05)

Table 4: Mean Density of Adult Mosquito species Collected within sites

Site	Mean Density±SD
Baba ruga	0.780±0.382
Darmanawa	1.000±0.229
Wudil	0.571±0.148

Table 5: Mean Density of Adult Mosquito species Collected within Rainy and Non-rainy Period

Season	Mean Density±SD
Dry	0.694±0.221[a]
Rainy	1.362±0.423[b]

Mean±standard deviation with the same letter within the column are not significantly different from each other (Least Significant Difference P<0.05)

DISCUSSION

The occurrence of 21 species of mosquitoes from 5 genera among the studied communities is an indication that these communities encourage their life cycle. Other techniques for mosquito identification will not stand without proper morphological identification in the first place. Darsie and Moris (2000) mentioned that identification of mosquitoes using morphological traits is the common method. Our findings show the worthiness of morphological characters for the correct identification of mosquito species. Oguoma and Ikpeze (2008) identified some similar species of mosquitoes from outdoor collections to including *Culex pipiens*, *Culex quinnquefasciatus*, *Mansonia* sp, *Psoropora* sp, *Anophele gambiae* and *Anopheles Funestus* from Gezawa and Jogana in Kano State. Also, Haruna *et al*. (2020) reported *Anophele gambiae*, *Anopheles Funestus* and Culex *quinnquefasciatus* from Aminu Kano Teaching Hospital. The species from the genera *Mansonia* and *Anopheles* except *Anopheles nigerrimus* showed larger dissimilarity and developed distinct cluster on the NJ tree. The *An. nigerrimus* displayed a divergent evolution among the *Anopheles* genera. The NJ tree also clearly separated the mosquitoes into different clusters of sub-families Anophelinae and Culicinae.

The high density of *An. gambiae* highlighted the high number of the female that emerged when the environmental condition is favorable following the wide distribution of breeding site among the communities. *An. gambiae* are known vectors of malaria, this conformed to the report of Visa *et al.* (2020) that, Kano State was selected for a study due its high prevalence of malaria (32%).

High density of female mosquitoes recorded in the months of November, August and September in this present study could be attributed to the temporal distribution pattern of these species. Similarly, Irikannu *et al*. (2023) point out that the decreasing population of adult mosquito in the month of August ahead of its climax in September. Mosquitoes displayed fluctuation in size annually, as such they are extremely temporal dynamics (Koenraadt *et al*., 2004; Whittaker *et al*., 2022). The high outdoor resting female mosquitoes as compared with indoor in this study could be associated with the modification from endophily to exophily among highly endophilic vector species in reaction to control measures intervention (Kreppel *et al*., 2020). The similarity in the density of female mosquitoes among the studied communities was in contrast with the findings of Ebenezer *et al*. (2013) and Afolabi *et al*. (2006) who reported variations in mosquito abundance across studied locations. This could be due to the similarities in environmental parameters of temperature and humidity among the locations in Kano State. The high density of female mosquito during rainy season is comparable to similar findings of Irikannu *et al*. (2023) reported a positive relationship between mosquito populations and rainfall (r=0.897). This could be attributed to increasing breeding sites formed by the rainfall.

CONCLUSION

Based on the finding from the studies, female mosquitoes density are similar among sites but varies within species, months of the year, resting behavior and rain period, because their number is affected by these variables. The high density of *An. gambiae* and *Cx. quinnquefasciatus* is bio-portent of malaria and filariasis spotlight in these communities. Further studies are needed to assess the abundance of the female mosquitoes.

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