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Research Article

Investigations on the Association of Mosquito Vector Species with Selected Tree Species in Nigerian Defence Academy, Ribadu Cantonment, Kaduna

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ABSTRACT

The study aimed to investigate the association of mosquito vector species with selected tree species at the Nigerian Defence Academy Ribadu Cantonment Kaduna. The study design was a Cross-sectional study primarily for distribution and relative abundance assessments. The study was carried out at the Nigerian Defence Academy Ribadu Cantonment, Kaduna, Nigeria. Mosquito samples were collected from the environment of tree species of interest using a mix of methods, as observed from several literatures. These include a yeast and sugar solution trap, a locally constructed hand-net, water-filled containers, and an ovipositing container. Following the mosquito collection on individual trees, physical and molecular identification were done using standard procedures. This was then succeeded by data analysis using Microsoft Excel 2016; descriptive statistics was used for summaries of the data and ANOVA, Chi square test were used to make inferences. A total of 2383 mosquitoes were collected from 140 tree samples of 10 different species. All the tree samples had different numbers of mosquitoes collected, with a variation in species. *Terminalia ivorensis* had the highest number of mosquitoes (631), and *Eucalyptus camaldulensis* had the lowest number (74). Using the Shanon Weiner Index calculator, the study was able to analyze the richness and evenness of mosquitoes to trees in the Cantonment. There is a significant relationship between different tree species and mosquito vectors within the Cantonment, and the degree to which mosquitoes are attracted to the tree species is based on specific characteristics of the tree species.

Keywords: Mosquitoes; Phytophagy; Phytotelmata; Vectors; Vegetation

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INTRODUCTION

Mosquito-borne diseases emerge as prominent causes of human and animal ailments and fatalities worldwide (Ohia *et al*., 2015). Several of the most epidemiologically significant mosquito-borne viruses pose a major threat to approximately 3.9 billion individuals across more than 120 countries (Shragai *et al*., 2017). According to the World Health Organization (2024), there were 263 million estimated cases of malaria globally in 2023, with an incidence of 60.4 cases per 1000 population at risk. This is an increase of 11 million cases from the previous year and a rise in incidence from 58.6 cases per 1000 population at risk in 2022. Globally, in 2023, the number of deaths caused by mosquitoes was estimated at 597,000, with a mortality rate of 13.7 per 100,000. The WHO African Region continues to carry the heaviest burden of malaria, accounting for an estimated 94% of malaria cases and 95% of malaria deaths worldwide in 2023; 76% of all deaths in this region were among children under 5 years (WHO, 2022). Presently, about 3,601 mosquito species have been cataloged and acknowledged worldwide, representing the diverse and medically significant Culicidae family (Azim *et al.,* 2023). The most pertinent man-biting mosquitoes hail from the genera *Anopheles, Culex, Aedes, Mansonia, Heamagogus, Sabethes,* and *Psorphora*. In Nigeria, studies have identified various mosquito species from three genera: Anopheles, Culex, and Aedes (Ogbuefi *et al*., 2023). The infections caused by mosquitoes encompass diseases such as Malaria, Chikungunya, Dengue fever, Yellow fever, Japanese encephalitis, Rift Valley fever, Filariasis, and West Nile diseases (Olagunju, 2023). Among these diseases, malaria and arboviruses like dengue, yellow fever, West Nile, and chikungunya are of considerable public health concern due to disease outbreaks, overwhelmed health systems during epidemics, and high morbidity and mortality rates in endemic regions (Freitas *et al*., 2012). The threat posed by mosquito-borne diseases continues to escalate, and current countermeasures, mostly vector control strategies are inadequate, necessitating urgent implementation of novel and efficient measures against mosquito-borne diseases (Shi *et al*., 2023). One such measure is the Global Vector Response program; in 2017, the World Health Assembly endorsed the Global Vector Control Response 2017–2030 and adopted a resolution to promote an integrated approach for the control of vector-borne diseases. The ultimate objective of the Global Vector Control Response is to mitigate the burden and threat of vector-borne diseases through effective, locally adapted, sustainable vector control measures in alignment with Sustainable Development Goal 3.3 (Global Vector Control Response, 2020). Studies into mosquito ecology reveal that the distribution and abundance of mosquito vectors largely depend on climatic factors such as rainfall patterns, temperature, and relative humidity (Irikannu *et al*., 2023). Vegetation, especially trees, has been said to play a critical role in the distribution and abundance of mosquito vectors by providing habitats for breeding and survival. Given that adult males of some species exclusively feed on plant sugar and females nest in various niches. Mosquitoes may therefore be said to exhibit a complex inter-relationship with fauna and environment, and the entire ecosystem ultimately affects their distribution and abundance (Kilpatrick *et al*., 2006). Understanding this relationship (especially those with trees and fauna) along with how it affects mosquito proliferation is necessary to proffer novel strategies for mosquito control.

MATERIALS AND METHODS

Study Area

The study was conducted at Ribado Cantonment (10°33'26.2"N 7°25'52.0"E), Nigerian Defence Academy Kaduna, Kaduna State, Nigeria. The area has an annual rainfall ranging from 900 mm to 1,500 mm, typically spanning between May and October, with a peak in August. The cantonment falls within the Northern Guinea Savannah, characterized by a mixture of grassy plains interspersed with scattered trees and shrubs. The Guinea Savannah woodland may attain a height of about 10 to 15m when fully developed and may be dense enough to suppress the growth of grasses (Yayock *et al.,* 2021). Grasses are mostly perennial, with species such as *Andropogon gayanus and Hyparrhenia spp*. dominating. The dry season lasts from November to April, characterized by the harmattan dry, dusty wind blowing from the Sahara Desert. Average temperatures range from 25°C to 35°C, though daytime temperatures can sometimes exceed 40°C during the peak of the dry season. The soils in the area are ferruginous tropical soils, which are relatively sandy with moderate organic content. They support savannah vegetation but may require supplementation for intensive agriculture. The region supports a variety of wildlife, including small mammals (e.g., rodents), reptiles, and birds (e.g., guinea fowl, hornbills). Larger mammals are rare due to urbanization. The natural vegetation is under pressure from human activities, such as military training exercises, construction, and urban sprawl. The cantonment includes developed areas for training, residential quarters, and infrastructure. The remaining undeveloped areas often consist of natural savannah and reforested patches.

Study Design

The Cross-sectional survey, used simple random sampling method to identify tree species with characteristics of interests for mosquito indigenization.

Sampling

A Simple Random Sampling (SRS) method was used to select tree species for this study. SRS is a widely accepted ecological sampling technique that ensures unbiased selection and represents various species across the study area (Thompson, 2012).The selection criteria included:

Canopy Cover: A sufficient layer of leaves, branches, and tree stems that provides shade and supports biodiversity (FAO, 2020).

Height: Trees taller than 6 meters (≥20 feet) were selected, as this height threshold aligns with standard tree classification in ecological studies (FAO, 2020).

Presence of Structural Features: Trees with cracks and crevices on their trunks were included due to their potential role as mosquito resting sites (Mutero *et al*., 2004).

Ecological Considerations: Trees frequently found near human residences and those known to attract mosquitoes were prioritized (Okech *et al*., 2007). Following standard botanical research practices, representative voucher specimens were collected and deposited at the Nigerian Defence Academy Herbarium, ensuring proper documentation and future verification (Bridson & Forman, 1998).

Tree Identification Procedure

Tree species were identified using artificial intelligence-based applications: PlantNet (version 3.16.2, developed by PlantNet Consortium, released July 13, 2023) and Google Lens (version 1.14.2203230, developed by Google LLC, released March 23, 2022). These tools have been validated for plant species identification, achieving over 80% accuracy in some studies (Wäldchen & Mäder, 2018). To ensure credibility, preliminary results from these applications were reviewed by a professor of Botany. The geographical coordinates of each sampled tree were recorded using Google Maps (version 11.65.0501, developed by Google LLC, released on December 15, 2023). Research indicates that smartphone GPS accuracy can range from 5 to 30 meters, depending on the device and conditions. The recorded GPS coordinates facilitate spatial mapping and precise documentation of tree species within the study area.

Mosquito sampling

Mosquito samples were collected from each tree environment for a period of 4 weeks to identify mosquito species per tree. Samples were collected during the early hours of the day and night times, specifically; 2 hours before sunset and 2 hours after sunrise (5:00pm – 7:00pm in the evenings and 6:30: 8:30 am in the morning). Sample collections during these time has been adopted in many recent vector surveillance studies to ensure that sampling methods are consistent with best practices (Service, 2018). Mosquitoes were collected using a combination of methods. The employed methods were to ensure successful collection at the different locations and developmental stages. These included: Yeast and Sugar Solution Traps: These traps utilize yeast fermentation to produce carbon dioxide, attracting mosquitoes (Rosanti *et al*., 2017) (Aizoun *et al*., 2022), Locally Constructed Hand-Nets: Hand-nets are commonly used for capturing adult mosquitoes in field conditions and remain an effective tool for active mosquito surveillance (Wamae *et al*., 2021). Water-Filled Containers: Simulating natural breeding sites, water-filled containers attract gravid females for oviposition. Research has shown that these trap designs, including tires and artificial containers, are effective in capturing immature mosquitoes (*Anopheles* and *Aedes* species) (Meyabeme *et al*., 2023). Following the mosquito collection on individual trees, mosquitoes were identified physically with the aid of a microscope using morphological characters of the keys of Gilles and Coetzee (1987), Medical Entomology for Students by Mike Service (5th Edition, 2012), and Photographic Guide to Common Mosquitoes of Florida (Cutwa & O'Meara, 2007). This was then succeeded by molecular characterization using standard DNA barcoding techniques, specifically Polymerase Chain Reaction (PCR) amplification of the cytochrome c oxidase subunit I (COI) gene, as described by Hebert *et al.* (2003). All mosquitoes collected were identified at the Nigerian Defence Academy Post Graduate School laboratory. Physical identification using Keys was done for morphological features before proceeding to molecular characterization and DNA barcoding.

Identification of collected mosquito species

Preliminary observations of the mosquitoes collected was done with the aid of a microscope and the mosquitoes were categorized them into 3 groups, based on their features. This was supported by the guidelines recorded in the keys of Gilles and Coetee (1987), medical entomology by Mike Service, and photographic guide to common mosquitoes of Florida by Cutwa (2017) and O'Meara (2012) based on morphological feature such as wing pattern, palps and proboscis, body color, nature of the antennae, and the marking on the thorax as used by Onodua *et al.,* (2020). Following this specimens were selected from the 3 categories for molecular identification. DNA was then extracted from each representative specimen using a commercial kit, Qiagen DNeasy Blood & Tissue Kit, and the established protocols were followed that optimize both yield and purity (Hebert *et al.,* 2003). The DNA was isolated, and the mitochondrial cytochrome c oxidase subunit I (COI) gene was targeted for PCR amplification, as this gene serves as a reliable DNA barcode for insects. The LCO1490 and HCO2198 primers were employed to amplify a fragment of approximately 650 base pairs, a region that is highly informative for species-level identification (Hebert *et al*., 2003). The PCR products were purified to remove excess primers and other reaction components, before preparing for Sanger sequencing. The resulting nucleotide sequences were edited to remove any low-quality regions, and these refined sequences were compared against reference databases GenBank to accurately determine the species identity (Kumar *et al*., 2018).

Data Analysis

The data was statistically analyzed using ANOVA and CHI square tests to observe if there was a statistical significant relationship; between tree species and mosquito species, composition and abundance using Microsoft Excel 2016 software. Descriptive statistics was used to get the summaries of the data while inferential statistics (ANOVA and Chi Square) were used to conclude. Shannon Weiner index for species diversity and abundance was used to calculate the abundance of mosquito species around the tree for this study. The index is

commonly used in ecology to quantify the diversity of a community, considering both the number of species (richness) and their relative abundance (evenness). The Shannon-Wiener Index (H') is calculated as: H'= -∑(pi x 1npi)
Where:
pi is the proportion of individuals belonging to the species is relative to the total number of individuals in the community.
The sum is over all species.
The formulas include:
For total abundance of mosquitoes:
N = Total number of mosquitoes
To calculate the Shannon-Wiener Index, we first need the proportion of each species out of the total collected mosquitoes: For the proportion of each tree species (i): Number of mosquitoes collected from tree species;
Pi = (Number of mosquitoes collected from tree species (i))/(Total number of mosquitoes)
Calculate pi ×ln (pi) for each species
Sum up all the pi ×ln (pi) values: ∑(pi x 1npi)
Calculate the Shannon-Wiener Index (H'): Take the negative of the sum above.

RESULTS

Fig 1 shows the mosquito abundance on selected trees. *Terminalia ivorensis* emerged as the tree species harboring the highest number of mosquito vectors, 631 mosquitoes, closely followed by Albizia lebbeck, which hosted a total of 330 mosquitoes, and lastly *Azadirachta indica* with 309 collections.
Table 1, shows the abundance of mosquitoes collected from a total of 140 tree samples. An abundance of 2383 was gotten for the mosquito collections. There was a variation of mosquitoes collected among the trees with *Terminalia ivorensis* commonly known as Black Afara, having the highest number of mosquitoes (631), followed by *Albizia lebbeck* (330), *Azadirachta indica* (309), *Delonix regia* (250), *Terminalia cattapa* (220) and *Alfizia Africana* (217) with all others having less than 200 collection and *Eucalyptus camaldulensis* commonly known as river red gum, having the lowest number of mosquitoes (74).
Table 2 shows the diversity in mosquito species collected around the tree species. All the tree species had a combination of both *Anopheles gambiae* and *Aedes aegypti*. Most of the trees also had significant numbers of *culex quinquefasciatus* along with the species of mosquito mentioned above, except for *Khaya senegalensis* and *Magnifera indica*.
Table 3 shows the Shannon Weiner index for each tree which shows the diversity of mosquitoes for each using the Shannon Weiner Calculator. The diversity value reflects the variety and the species richness of mosquito species populations across different tree species. The Shannon-Wiener Diversity Index for each of the tree species was less than 1, which means that the diversity for each tree is low, but all add to the overall score for the selected trees in the study site.
Fig 2, show the mosquitoes identified at the Ribadu Cantonment. The species of mosquitoes gotten around the study area *include Anopheles gambiae, Culex quinquefasciatus* and *Aedes aegypti*. The mosquitoes identified were discovered to be 24% Aedes spp, 30% Culex spp and 46% Anopheles spp.

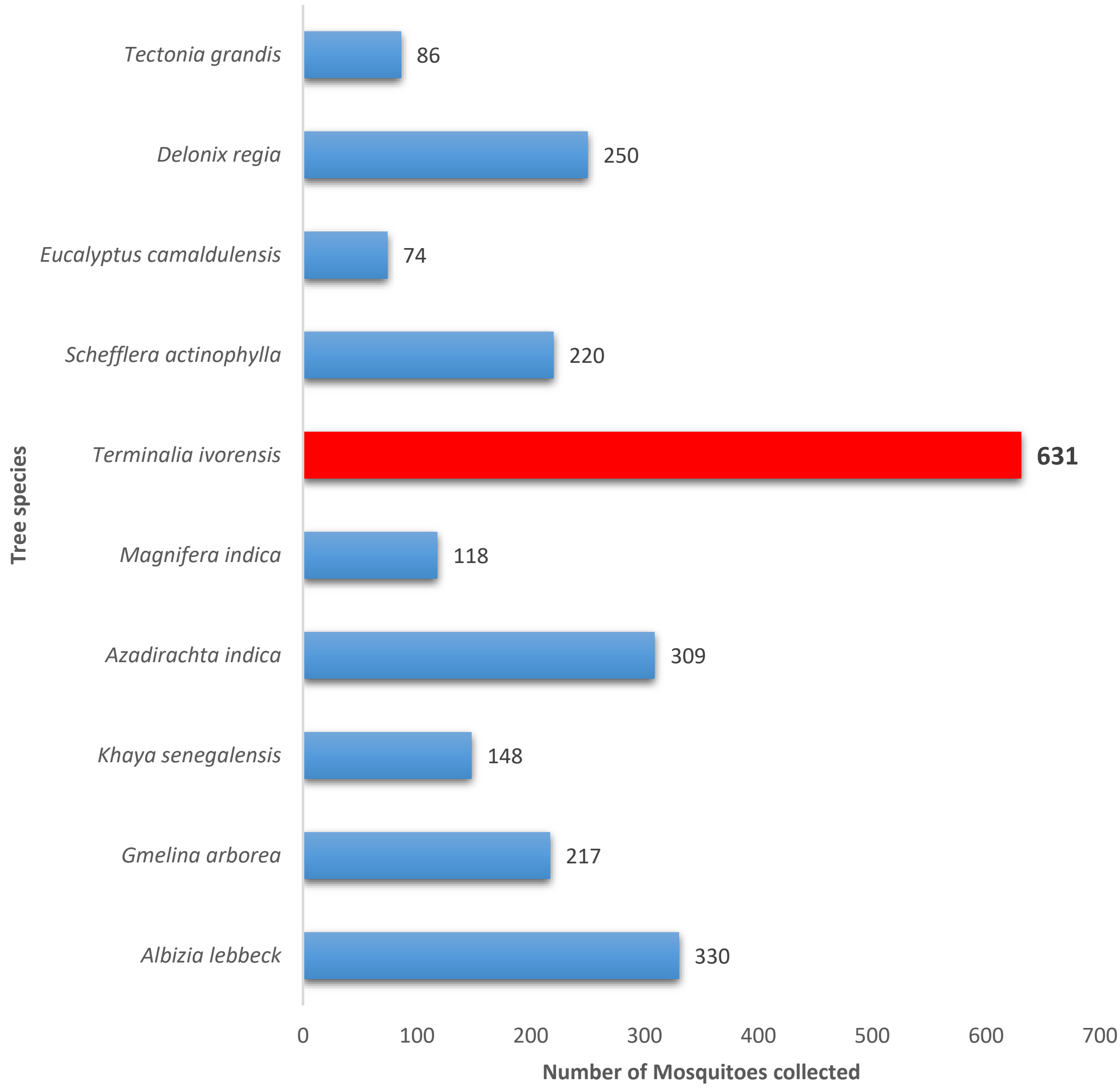

Fig. 1. Showing the mosquito abundance on selected trees

Table 1: the diversity in species of mosquitoes associated with selected tree species

Tree Species	**Number of Trees Sampled**	***Anopheles gambiae***	***Culex quinquefasciatus***	***Aedes aegypti***
Albizia lebbeck (Woman's Tongue Tree)	14	180	127	23
Gmelina arborea (African Rosewood)	14	106	75	36
Khaya senegalensis (African Mahogany)	14	140	0	8
Azadirachta indica (Neem)	14	132	77	100
Mangifera indica (Mango)	14	80	0	38
Terminalia ivorensis (Black Afara)	14	235	329	67
Terminalia catappa (Umbrella)	14	100	10	110
Eucalyptus camaldulensis (Eucalyptus)	14	11	51	12
Delonix regia (Flamboyant)	14	109	11	130
Tectona grandis (Teak)	14	8	35	43

Table 2: Shannon Weiner Index for mosquito diversity for each tree

Tree species	Mosquito collection	Shannon Weiner Index
Albizia lebbeck	330	0.138
Gmelina arborea	217	0.091
Khaya senegalensis	148	0.062
Azadirachta indica	309	0.130
Mangifera indica	118	0.050
Terminalia ivorensis	631	0.265
Terminalia catappa	220	0.092
Eucalyptus camaldulensis	74	0.031
Delonix regia	250	0.105
Tectona grandis	86	0.036

Table 3: Shannon Weiner Index for mosquito diversity for each tree

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Terminalia ivorensis - Black Afara	631	0.265
Terminalia catappa - Umbrella	220	0.092
Eucalyptus camaldulensis - Eucalyptus	74	0.031
Delonix regia - Flamboyant	250	0.105
Tectona grandis - Teak	86	0.036

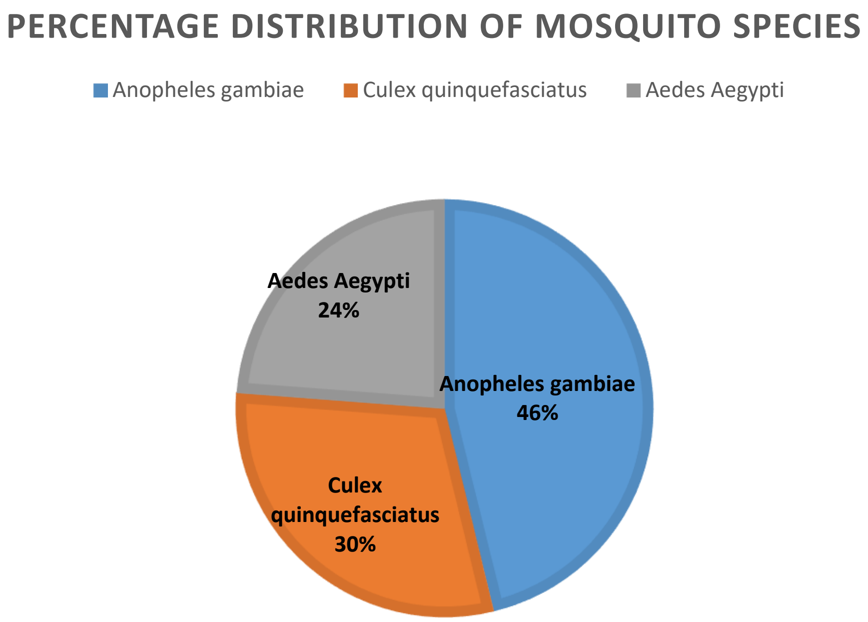

Fig. 2: show the mosquitoes identified at the Ribadu Cantonment

DISCUSSION

Malaria and other mosquito-borne diseases continue to pose global threats, affecting over one million people annually (World Health Organization, 2023). Mosquito vectors are specific to illnesses, and the spread of mosquito-borne diseases in a region is largely driven by the predominant mosquito species inhabiting it (Siddiqui & Kayte, 2022). Research indicates that trees play a crucial role in mosquito ecology. The findings of this study demonstrate a significant relationship between trees and mosquito populations within the study site. The 2,383 mosquitoes collected in this study show statistically significant variations in abundance across different tree species within Ribadu Cantonment. The three distinct mosquito species identified *Anopheles gambiae* (1,101 mosquitoes, 46.2% of the total), *Culex quinquefasciatus* (715 mosquitoes, 30.0% of the total), and *Aedes aegypti* (567 mosquitoes, 23.8% of the total) yielded a Shannon-Wiener Diversity Index of 2.11, suggesting a moderate level of diversity. Variation in mosquito abundance among tree species is influenced by factors such as canopy cover, phytotelmata, resting/breeding sites (e.g., tree holes), and nutritional sources. These findings align with Mangudo *et al*. (2017), who observed that mosquito exhibit strong associations with trees and that urbanization negatively impacts mosquito diversity. Anosike *et al*. (2007) reported that mosquito abundance and species composition varied across biotypes, including natural tree holes and bamboo traps. Similarly, Rogers *et al*. (2023) determined that tree canopy cover influences the nutrient profiles of mosquito larvae, affecting their development and fecundity. Mosquito attraction to trees is significantly influenced by their phytophagous behavior, as plant-derived sugars are essential for survival and reproduction. Peach & Gries (2020) noted that newly emerged mosquitoes rely on sugary plant liquids for energy. Schlein & Müller (2008) found that certain trees attract mosquitoes due to their blossoms, which provide a food source. The high mosquito abundance observed suggests a thriving population, while the diversity indicates that not all trees contribute equally to proliferation. Environmental factors such as tree type, structure, and ecological interactions shape mosquito attraction. Tree characteristics including foliage, shade, humidity, nectar, fruit, and essential oils enhance or reduce mosquito presence. Overall, tree-associated habitats sustain mosquito biodiversity, influencing ecological balance and disease transmission (Hutchings *et al*., 2011). Terminalia ivorensis harbored the highest number of mosquitoes (631), followed by *Albizia lebbeck* (330) and *Azadirachta indica* (309). This suggests that different tree species vary in their suitability for mosquito attraction. *Terminalia ivorensis*, characterized by its trunk crevices, tree holes, and dense canopy, may enhance mosquito resting/breeding potential. The textured bark also aids in camouflage, improving mosquito survival (Kang *et al*., 2013; Wang & Schaefer, 2012). Certain tree species serve as breeding and resting sites due to leaf litter, shade, and nectar sources (Foster, 1995; Clements, 2012). However, urbanization disrupts these habitats, increasing predation risks. Yang *et al*. (2023) found that tree coverage, particularly shrubs, plays a critical role in mosquito abundance, accounting for 55.2% of observed variance in urban parks. Farner *et al*. (2024) determined that local tree cover predicts mosquito species richness, with more diverse communities occurring in areas with higher tree cover. Mosquitoes, particularly Anopheles species, are attracted to plant volatiles, including those from *Terminalia ivorensis*. Studies show that specific volatile compounds from graminoid plants attract gravid *Anopheles gambiae*, influencing oviposition site selection (Bokore *et al*., 2021). Synthetic odor blends derived from natural grass volatiles effectively lure mosquitoes, suggesting that *Terminalia ivorensis* may release similar compounds (Wondwosen *et al*., 2021). Understanding these interactions is crucial for mosquito control strategies leveraging plant volatiles to attract and trap vectors (Kemibala *et al*., 2020; Bokore *et al*., 2021).

The presence of Anopheles, Culex, and Aedes species in the study area aligns with research indicating that mosquito attraction to trees is influenced by plant volatiles and biodiversity. *Anopheles gambiae* is drawn to local fruits and flowering plants, including guava and honey melon (Müller *et al*., 2010). Similarly, Acacia macrostachya attracts both male and female *Anopheles gambiae,* emphasizing the role of floral scents (Müller *et al*., 2010). Increased tree cover correlates with greater mosquito species richness (Farner *et al*., 2024). Gravid *Culex quinquefasciatus* and *Aedes aegypti* are attracted to volatiles from aquatic plants like water hyacinth, which stimulate oviposition (Turnipseed *et al*., 2018). Vegetation types including trees and aquatic plants significantly shape mosquito behavior. These species transmit malaria, lymphatic filariasis, and dengue fever (Okorie *et al*., 2011), indicating potential disease risks in Ribadu Cantonment. Mosquitoes are also drawn to specific chemicals emitted by trees and plants, known as kairomones, which influence mosquito behavior and host-seeking strategies. Some kairomones act as phytochemical attractants luring mosquitoes of all ages and sexes (Foster, 2008). Esters and chlorohydrins have been identified as effective attractants for female *Aedes aegypti* (McGovern *et al*., 1970). However, species-specific attraction and competition from other volatiles may limit broad applications (Shajahan *et al*., 2022). Understanding these attractants can aid in developing effective mosquito control measures.

CONCLUSION

This study confirms a significant relationship between mosquito abundance and diversity across different tree species in Ribadu Cantonment. All sampled tree species exhibited some level of mosquito association, influenced by unique tree characteristics. Given these findings, it is crucial to recognize trees' impact on mosquito vector populations. Proactive measures should be considered when planting tree species, especially *Terminalia ivorensis*, which demonstrated a high mosquito attraction rate. Such insights can inform effective mosquito control strategies to mitigate disease risks associated with mosquito-borne infections.

Given the mosquito abundance in trees such as *Terminalia ivorensis*, it is recommended that targeted vector control interventions, such as insecticide spraying or larval source management, be prioritized around these tree species. Additionally, the incorporation of trees with mosquito-repellent properties, like *Eucalyptus camaldulensis*, in landscape planning within mosquito-prone areas may help reduce vector populations. Tree interactions and relationships should be considered when planting trees around residential areas. Further molecular studies should be conducted to precisely identify the species related relationship between trees and mosquitoes present and their disease transmission potential.

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