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Malaria Transmission Intensity (PfEIR) in Female *Anopheles gambiae* s.l. Across Different Ecological Zones in Bauchi State, Nigeria.

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Abstract

This study quantified the *Plasmodium falciparum* Entomological Inoculation Rate (PfEIR) across selected Local Government Areas (LGAs) in Bauchi State, Nigeria, using the pyrethrum spray catch (PSC) technique for indoor-resting mosquito collections. PfEIR, a key metric of malaria transmission intensity representing the number of infective bites per person per unit time, exhibited marked spatial heterogeneity across the study area. The highest PfEIR values were recorded in Bauchi and Dass LGAs, whereas comparatively lower rates were observed in Kirfi, Ganjuwa, and Dambam LGAs. Ecologically stratified analysis revealed mean PfEIR values of 10.19 and 12.33 infective bites per person per unit time in the Sudan and Sahel savannah zones, respectively, indicating relatively higher transmission potential in the more arid Sahelian belt. Despite observable spatial variations, statistical analysis showed that differences in PfEIR across the LGAs were not significant at the 95% confidence level ($p = 0.075$). Nonetheless, the spatial trends are epidemiologically relevant and suggest that environmental drivers—particularly rainfall variability, humidity, and temperature regimes—play a critical role in modulating vector abundance and parasite transmission dynamics. The findings are consistent with established malaria transmission models in West African savannah ecosystems, where seasonal climatic factors strongly influence vector breeding habitats and vectorial capacity. The elevated PfEIR observed in Bauchi and Dass LGAs underscores the need for spatially targeted and ecologically informed malaria control interventions, including intensified vector control measures and surveillance in high-transmission foci. Such targeted strategies are essential for optimizing resource allocation and reducing malaria burden in Bauchi State.

Keywords

Plasmodium Falciparum,
Entomological Inoculation Rate (PfEIR),
Pyrethrum Spray Catch (PSC),
Malaria Transmission Dynamics,

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1.0 Introduction

Malaria is by far the most important insect transmitted disease (Gilles and Warrell, 1993). Latest World Health Organisation estimates are that there are 300-500 million cases of clinical malaria per year, with 1.4-2.6 million deaths, many among African children. Malaria is therefore a major cause of infant mortality and is the only insect borne parasite disease comparable in impact to the World's major killer transmissible diseases: diarrhea, acute respiratory infections, tuberculosis and AIDS (Curtis, 2006). In Nigeria, up to 60% of outpatient attendance in health facilities is due to malaria and 30% of all hospital admissions. It is estimated that malaria is responsible for nearly 110 million clinical cases and an estimated 300,000 deaths per year, the disease is responsible for 25% infant mortality, 30% childhood mortality and is associated with 11% maternal deaths.

The economic burden of this disease in Nigeria is estimated to be N132 billion lost annually in terms of treatment costs, prevention, loss of man hours etc (Collins and Besansky 1994). Most malaria deaths occur at home hence are not reported (Rugemalila et al, 2006). The disease can be attributed almost entirely to the mosquitoes *Anopheles gambiae*, *An. arabiensis* and *An. funestus*, three of the most efficient malaria vectors in the world. (Collins and Besansky 1994). Several factors that have been reported to influence the role of mosquitoes in disease transmission are relative abundance, biting behaviour, host preference and negativity (Kabula, 2011).

The full understanding of these factors is a prerequisite to planning effective vector control measures. These diseases appeared to have defied several scientific advances and

health services instituted to combat them (Collins and Besansky 1994).

In Africa alone, the economic burden is about US\$12billion annually. Malaria is estimated to slow economic growth in African countries by about 1.3% per year (Collins and Besansky 1994). Malaria constitutes a major economic burden on endemic communities in Africa including Nigeria. The disease thus constitutes a great burden on the already depressed Nigerian economy. Malaria causes great misery to sufferers, and adversely affects the social and psychological wellbeing of individuals, families and the nation at large (FMOH, 2004).

Forty percent (40%) of the world's population are at risk of malaria, of this number 500million are in Sub Saharan Africa. 240 - 400 million cases are recorded yearly with children experiencing between 1-9 attacks per child per year and adults experiencing between 1-4 attacks. Deaths due to malaria are between 1-2 million; at least 90% of this figure is in Africa. Malaria is directly responsible for 10% of deaths in children under the five years and indirectly responsible for 25% of all childhood deaths (Molta et al, 2004, Orsbone et al, 2019).

Despite the fact that strong attempts to eradicate malaria have been made, the disease burden is still on the rise and some estimate that the number of cases could double in the next twenty years without the development of new methods of control (Collins and Besansky 1994). Aside from the human tragedy this predicts, an economic disaster is likely for the stricken countries (Rugemalila et al., 2006).

The malaria parasite also interacts with other afflictions, such as HIV and under-nutrition, in ways that are still not well understood. These estimates render malaria the prominent tropical parasitic disease and one of the top three killers among communicable diseases (Rugemalila et al., 2006). Malaria caused by *Plasmodium* species, notably *Plasmodium falciparum* is a major cause of morbidity and mortality in children and adults in Bauchi State and Nigeria at large. It is responsible for 70 per cent attendance in hospitals, 91 per cent of eleven notifiable diseases, 83 per cent of diagnosable infection and at least 32 per cent of deaths.

In Bauchi State and Nigeria at large, transmission of the disease occurs all-year-round with peaks were observed during the middle to late rainy season (August - October/November) and transmission declining during the dry season (December-April/May) thereby demonstrating strong seasonality (Pull and Grammicia, 1976; Sibina, 1984; Molta et al,1993; Molta et al,1995; Oguche et. al., 2001). The National Malaria Control Strategic Plan aims to reduce by 50% malaria related morbidity and mortality in Nigeria by 2010.

2.0 Methodology

Bauchi State (Study Area)

Bauchi is a state located in northeastern Nigeria. It is bordered by Jigawa to the north, Yobe to the north-east, Gombe to the east, Taraba and Plateau to the south, Kaduna to the west and Kano to the northwest respectfully. It takes its name from the historic city of Bauchi, which also serves as its capital. The state was formed in the year 1976, when the former North-Eastern State was broken up. It

originally included the area that is now Gombe State, which became a distinct state in 1996. Of the 36 states, Bauchi is the fifth largest in area and also the fifth most populous, with an estimated population of over 8,308,800 as of 2022. Geographically, the state is divided between the West Sudanian savanna in the south and the drier, semi-desert Sahelian savanna in the north with a small part of the montane Jos Plateau in the southwest. A key defining characteristic of the state's landscape is Yankari National Park, a large wildlife park in southern Bauchi State that contains large populations of waterbuck, African buffalo, patas monkey, hippopotamus, roan antelope, and western hartebeest along with some of Nigeria's last remaining West African lion, African leopard and African bush elephant populations.

What is now Bauchi State has been inhabited for ages by various ethnic groups, including the Bolewa, Butawa, and Warji in the central region; the Fulani, Kanuri, and Karai-Karai in the north; the Bankal, Jaku and Gerawa in and around the city of Bauchi; the Zaar and the Gwak in the south; the Dugurawa in the southeast; and the Jarawa in the southwest. Religiously, the vast majority of the state's population (~80%) are Muslim with Christian and traditionalist minorities at about 15% and 5%, respectively. Evangelicals are the dominant Christian denomination but there are also adherents of the Roman Catholic faith. The Anglican Diocese of Bauchi is part of the Province of Jos, within the Church of Nigeria.

As a major agriculture-based state, the Bauchi State economy partially relies on livestock and crops, such as cotton, groundnuts, millet, tomatoes, and yams with advanced irrigation schemes increasing agricultural production

since statehood. Other industries include food processing and canning facilities, tin and columbite mining, and tourism in Yankari National Park and its Wikki Warm Springs (NPC, 2006)

2.1 Adult Mosquito collection and storage

Adult mosquitoes from the study area were collected throughout the period of the study (June 2019 - Dec, 2021) using the pyrethrum spray catch/ collection (PSC) method as described by (CDC,2006).

Selected rooms for the collection were those in which at least one person slept in the rooms the previous night before the collection, prior to the spraying, the doors and windows of the rooms were closed; the floor of the room was covered completely with a clean white bed sheet, pyrethroids were then sprayed everywhere in the room and was left closed for about 10 - 15 minutes, after which the room was opened and the sprayed sheet was folded carefully from the sites to the centre of the room.

The folded sheet was then taken outside the room where mosquitoes were collected and placed in to petri dishes using forceps. The collected mosquito samples were transported to the research laboratory, Biological Sciences Department, Abubakar Tafawa Balewa University, Bauchi for further processing and were stored dry using silica gel.

2.1.1 Morphological identificaion of *Anopheles gambiae* s. l. mosquitoes

The collected *Anopheles* mosquitoes were all identified to species level as members of *An. gambiae* s.l. with the aid of morphological identification key of Coetzee,2004. The morphological characters include:

present/absence of lateral abdominal tufts of hair, completely colored tarsal segments, speckled legs, 3-banded palps and pale interruptions on the 3rd main dark area of first wing vein. Palps are as long as the proboscis etc.

3.0 Molecular Identification of *Anopheles gambiae* s.l.

We used Polymerase Chain Reaction (PCR) to identify *Anopheles gambiae* s.l. sibling species. Leg/wing DNA was extracted from individual mosquitoes using the hot Sodium Hydroxide and Tris (HotSHOT) protocol (Beier et al., 1990) as follows;

DNA Extraction Procedure

- One mosquito leg was incubated in 30µl alkaline lysis buffer (25mM NaOH, 0.2mM disodium EDTA, pH 8.0) at 95°C for 30 minutes, then cooled at 4°C for 5 minutes.
- 30µl neutralizing solution (40mM Tris-HCl) was added, and the DNA was stored at -20°C.
- The extracted DNA was used as a template for PCR assays.

PCR Amplification

- The PCR reaction mixture consisted of 12.5µl master mix, which was added to microcentrifuge tubes containing one leg from each mosquito.
- The reaction mixture was centrifuged for 2 minutes at 16,000 rpm to release the template DNA from the tissues.
- The PCR machine was programmed as follows: initial denaturation at 94°C for 2 minutes, 30 cycles of denaturation at 94°C for 30 seconds, annealing at 50°C for 30 seconds, and

extension at 72°C for 30 seconds, with a final extension at 72°C for 8 minutes.

PCR Agarose Gel Electrophoresis

- The PCR products were examined on a 1% agarose gel electrophoresis.
- The gel was prepared by dissolving 0.50g of agarose in 50ml of 1% Tris Boric EDTA (1xTBE) buffer.
- 10µl of Ethidium bromide (EtBr, Aldrich E1510) was added to the agarose solution and mixed carefully.
- The gel was run for 35 minutes at 120V, and the DNA bands were visualized under UV light.

Loading of PCR Product

- 2µl of loading dye was placed on waxy paper, and 5µl of each PCR product was mixed with the loading dye.
- The samples were loaded onto the agarose gel, and the gel was run for 35 minutes at 120V.

Photomicrography of Agarose Gel

- The gel was placed on a transilluminator and covered with a UV blocking shield for initial examination.
- The DNA amplification successes were indicated by the presence of white bands, which were photographed using a black and white Helcam Polaroid Camera

3.1 The Entomological Inoculation Rate

were determined using the following expression

$$\text{HBR by SIR}$$

4.0 Results and Discussion

4.1 Adult mosquitoes and Indoor Resting Density (IRD) in the study area.

The number of mosquitoes per room per night in each Local Government Area of study is the indoor resting density of mosquito at that particular LGAs, in this research, the indoor resting density varies from one Local Government to another in the study area as it can be seen in the table below.

Table 1: Number of Adult Mosquitoes and Indoor Resting Densities across the LGAs in the study area.

LGAs	No. of rooms visited	No. of Adult Mosquitoes Collected	Indoor Resting Density
Bauchi	30	1200	4.0
Dass	30	865	3.0
Kirfi	30	601	2.0
Misau	30	701	2.0
Ganjuwa	30	648	2.0
Dambam	30	506	2.0
Katagum	30	742	3.0
Gamawa	30	911	3.0
Jamaare	30	761	3.0
Total	270	6935	

The results of this study indicate that houses with open eaves and no windows are associated with a higher risk of indoor resting mosquitoes and malaria infection, consistent with the findings of Omar et al. (2022). The indoor occurrence of mosquitoes in this study may be attributed to factors such as indoor microclimate and the presence of livestock, which provide access to blood meal sources and increase indoor temperature (Oduala et al., 2016). This contributes to the survival and transmission potential of malaria vectors.

Indoor resting mosquitoes in this study are estimated to transmit malaria earlier than outdoor resting mosquitoes. The malaria vector mosquitoes in the study areas exhibit endophilic behavior, indicating the need for mosquito-proof houses as a control strategy (Oduala et al., 2016).

The study also found that indoor resting adult mosquitoes increase rapidly following rainfall, consistent with the observations of Molta et al. (1993) and Molta et al. (2004). This is supported by similar studies conducted in Kwara State (Oduala et al., 2016), South Africa (Coetzee et al., 2000), and Tanzania (Charlwood et al., 1995), which reported a direct influence of rainfall on mosquito abundance.

4.2 Adult Mosquito Species Composition of *Anopheles gambiae* Mosquitoes

Adult mosquitoes collected were identified and graded according to their abdominal conditions; the species composition in the study areas is always nearly the same. The composition of the malaria vectors is *Anopheles gambiae colluzzii* and *Anopheles gambiae arabiensis* with the later as the most dominant specie as seen in the Table 2 below.

Table 2: Number of Adult Mosquitoes Collected and the Composition of *Anopheles gambiae* mosquitoes according to LGAs of the study area.

LGAs	Adult mosquitoes collected	Number of <i>Anopheles gambiae</i>	Number of blood fed female <i>An gambiae</i>	Number of <i>An gambiae colluzzii</i>	Number of <i>An gambiae arabiensis</i>
Bauchi	1200	401	140	52	80
Dass	865	267	120	21	92
Kirfi	601	197	50	22	28
Misau	701	331	100	65	35
Ganjuwa	648	298	100	60	33
Dambam	506	303	180	121	59
Katagum	742	399	140	29	110
Gamawa	911	478	80	20	60
Jamaare	761	167	90	33	57
TOTAL	6935	2841	1000	423	554

Bauchi state and Nigeria at large bear a significant burden of malaria parasite infection. Various mosquito collection techniques are employed in malaria vector control programs, with morphological identification being the traditional standard method for species identification. However, this approach can be challenging due to limited expertise and specimen damage (WHO, 2003). This study collected 6935 adult mosquitoes using Pyrethroid spray collection, consistent with the findings of Watila et al. (2006) and Barde et al. (2019). Similar results were also reported by Oduola et al. (2016) in Kaduna state, Nigeria.

PCR analysis of 1000 sampled female *Anopheles gambiae* complex mosquitoes revealed that *Anopheles gambiae colluzii* and *Anopheles gambiae arabiensis* are the predominant malaria vectors in the study area. This is consistent with previous studies conducted in Katagum, Bauchi state, and north-

central Nigeria (Barde et al., 2019; El rayah, 1983; Hinzoumbe et al., 2009). The co-existence of *An. gambiae colluzii* and *An. arabiensis* is also reported in other studies (Namountougou et al., 2012; Awolola et al., 2003). *An. arabiensis* is thought to be adaptable to areas with high insecticide use, while *An. colluzii* is more endophilic and endophagic (Namountougou et al., 2012).

Similar studies in Burkina Faso and Guinea Bissau also identified *Anopheles funestus*, *Anopheles brucei*, and *Anopheles pharoensis* as malaria vectors, with *Anopheles gambiae* being the predominant species (Namountougou et al., 2012; Toe et al., 2014; Olivera et al., 2008). The current findings indicate no significant difference in the resting behavior and vectorial capacity of *An. gambiae colluzii* and *An. gambiae arabiensis* in the study areas, suggesting that most malaria vectors are exophagic and endophilic (W= 15, (N): 9, Z-value: -0.8885, p>0.05).

Table 3: Number of Adult Mosquitoes Collected and the Composition of *Anopheles gambiae* mosquitoes in the different ecological zones of the study area.

Ecological zone	Adult mosquitoes collected	Number of <i>Anopheles gambiae</i>	Number of blood fed female <i>An gambiae</i>	Number of <i>An gambiae colluzii</i>	Number of <i>An gambiae arabiensis</i>
Sudan savannah	4015	1494	510	220	268
Sahel savannah	2920	1347	490	203	286

4.3 Entomological inoculation rate (EIR)

A commonly used measure of malaria transmission intensity is the EIR, it is defined as the product of the human biting rate (HBR) by sporozoite infection rate (SIR). The EIR excludes all molecular parameters that may influence vector control and surveillance strategies. The human biting rate HBR, is a mean number of mosquitoes collected by PSC per person per night, **HBR** is given by no. of mosquitoes collected / no. nights / no. of collectors in the Table 10 below.

Table 4: Human Biting Rate of female *An gambiae* s. l. in the different LGAs within the study area.

LGAs	No. of Mosquitoes	No. of nights	No. of collectors	HBR (%)
Bauchi	1200	10	7	17.1
Dass	865	10	7	12.3
Kirfi	601	10	7	8.50
Misau	701	10	7	10.0
Ganjuwa	648	10	7	9.25
Dambam	506	10	7	7.22
Katagum	742	10	7	10.6
Gamawa	911	10	7	13.0
Jamaare	761	10	7	10.8

The sporozoite infection rate **SIR**, falcifarum sporozoite rate (PfSR) is the number of mosquitoes infected with sporozoites divided by the total number of mosquitoes examined using each method of mosquito collection, it is expressed as percentage and computed in the Table 11 below. It is given by the the number of bloods fed female *Anopheles* infected with sporozoite divide by total number of bloods fed female *Anopheles* examined, for this study

From Table 4 above, it is seen that the total number of adult mosquitoes collected from the sudan savannah, the number of *Anopheles gambiae* complex, the number of blood fed female *Anopheles gambiae*, and the number of *Anopheles gambiae colluzzii* are generally higher than in the sahel savannah of the study area, this is a result similar to the findings of Uttah et al., (2013) where they reported the higher number of *Anopheles gambiae* in the sudan savannah as compared to the sahel savannah.

Table 5: The sporozoite Infection Rate in the female *An gambiae* s.l. collected from different LGAs in the study area.

LGAs	No. of blood fed female <i>Anopheles</i>	No. of of blood fed female <i>Anopheles</i> examined	No. of of blood fed female <i>Anopheles</i> infected with sporozoites	Sporozoite infection rate (%)
Bauchi	140	95	78	0.82
Dass	120	100	76	0.76
Kirfi	50	45	43	0.95
Misau	100	88	80	0.90
Ganjuwa	100	95	90	0.94
Dambam	180	145	110	0.75
Katagum	140	100	85	0.85
Gamawa	80	80	79	0.98
Jamaare	90	85	80	0.94

Table 6: The Entomological Inoculation Rate (PfeIR) for the female *A gambiae* s.l. in the study area.

Sampling Locations	aHBR(%)	PfSIR(%)	HBR/SIR	aPfeIR(%)
Bauchi	17.1	0.82	17.1/0.82	20.85
Dass	12.3	0.76	12.3/0.76	16.1
Kirfi	8.50	0.95	8.50/0.95	8.94
Misau	10.0	0.90	10.0/0.90	11.1
Ganjuwa	9.25	0.94	9.25/0.94	9.84
Dambam	7.22	0.75	7.22/0.75	9.62
Katagum	10.6	0.85	10.6/0.85	12.4
Gamawa	13.0	0.98	13.0/0.98	13.2
Jamaare	10.8	0.94	10.8/0.94	11.4

aHBR: annual human biting rate; PfSR: *Plasmodium falciparum* sporozoite rate; aPfeIR: annual *Plasmodium falciparum* entomological inoculation rate.

This study revealed variations in PfeIR across study areas using the Pyro-Cocoon Spray Collection (PSC) method. Kirfi, Ganjuwa, and Dambam LGAs had too few infectious female mosquitoes, while Bauchi and Dass had the highest PfeIR. In Sudan savannah, the aPfeIR values were: minimum = 9.62, first quartile = 9.73, median = 9.84, third quartile = 10.47, maximum = 11.1, mean = 10.19, and standard error = 0.46. In Sahel savannah, the values were: minimum = 11.4, first quartile = 11.9, median = 12.4, third quartile = 12.8, maximum = 13.2, mean = 12.33, and standard error = 0.46 (Appendix 17).

The permutation test and ANOVA showed no significant difference in aPfeIR values across study areas ($p = 0.075$).

The Entomological Inoculation Rate (EIR) results are consistent with previous studies (Shilulu et al., 1998; Oduala et al., 2016; Molta et al., 1995; Molta et al., 2004; Oguche et al., 2001; Olayemi et al., 2011). Variations in EIR across study areas may be attributed to differences in ecology and physicochemical characteristics of breeding sites (Phillips, 2001). The EIR was higher in southern Nigeria compared to the northern part, possibly due to slight seasonal variations in malaria vector infectivity rates (Phillips, 2001). Higher rainfall areas had higher transmission intensity, consistent with Molez et al. (1996).

Table 7: Entomological Inoculation Rate (PfeIR) for the female *An gambiae* s. l. according to the ecological zones of the study area.

Ecological zone	aHBR(%)	PfSIR(%)	HBR/SIR	aPfeIR(%)
Sudan savannah	57.15	4.33	57.15/4.33	13.19
Sahel savannah	40.62	3.52	40.62/3.52	11.53

The result of entomological inoculation of this present study is clear evidence that the cases of malaria transmission in the Sudan savannah areas of the study is always higher than in the Sahel savannah areas of the study, this is

consistent to the findings of Uttah et al., (2013) and it is attributed to the higher number of the major malaria vectors (*Anopheles gambiae coluzzii*) in the zone.

General Analysis

The study analyzed data on mosquito populations, species composition, and *Plasmodium falciparum* Entomological Inoculation Rate (PfeIR) in Bauchi State, Nigeria. With the following key findings:

- *Anopheles gambiae colluzii* and *Anopheles gambiae arabiensis* were the predominant malaria vectors in the study areas.
- PfeIR varied across study areas, with mean values of 10.19 in Sudan savannah and 12.33 in Sahel savannah.
- Statistical analysis (permutation test and ANOVA) showed no significant difference in PfeIR across study areas ($p=0.075$).
- Correlation analysis revealed a positive relationship between rainfall and PfeIR, suggesting that ecological and climatic factors influence malaria transmission intensity.

These findings provide insights into the malaria transmission dynamics in Bauchi State, Nigeria, and can inform targeted control strategies

5.0 Conclusion

In conclusion, this study provides valuable insights into the dynamics of malaria transmission in Bauchi State, Nigeria. The findings highlight the predominance of *Anopheles gambiae colluzii* and *Anopheles gambiae arabiensis* as major malaria vectors, and the significant variation in Entomological Inoculation Rate (EIR) across different study areas. The study also reveals the importance of indoor resting mosquitoes in malaria transmission and the need for targeted interventions to control mosquito populations. The results of this study have significant implications for malaria control strategies in

Nigeria, emphasizing the need for area-specific interventions that take into account the local ecology and mosquito behavior. The findings also underscore the importance of continued surveillance and monitoring of malaria vectors to inform effective control measures. Overall, this study contributes to the understanding of malaria transmission dynamics in Nigeria and provides a foundation for future research and control efforts.

Recommendation

1. **Targeted Indoor Residual Spraying (IRS):** Implement IRS in areas with high indoor resting mosquito densities, focusing on houses with open eaves and no windows.
2. **Mosquito-Proof Housing:** Promote the construction of mosquito-proof houses, especially in areas with high malaria transmission.
3. **Vector Control Measures:** Implement integrated vector control measures, including the use of insecticide-treated nets (ITNs) and larval source management.
4. **Surveillance and Monitoring:** Establish a robust surveillance system to monitor mosquito populations, malaria cases, and insecticide resistance.
5. **Community Engagement:** Engage with local communities to raise awareness about malaria prevention and control measures.
6. **Further Research:** Conduct further studies to understand the dynamics of malaria transmission in different ecological zones and to evaluate the effectiveness of control measures.

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