PMI VECTORLINK PROJECT

THE DEMOCRATIC REPUBLIC OF CONGO ANNUAL ENTOMOLOGICAL MONITORING REPORT

JANUARY–DECEMBER 2021

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ACRONYMS

CDC	Centers for Disease Control and Prevention
CI	confidence interval
CTAB	cetyltrimethylammonium bromide
DRC	Democratic Republic of Congo
EIR	entomological inoculation rate
ELISA	enzyme-linked immunosorbent assay
GTLAV	*Groupe de Travail Lutte Anti-Vectorielle* / Vector Control Working Group
HBR	human biting rate
HLC	human landing catch
INRB	*Institut National de Recherche Biomédicale* / National Institute of Biomedical Research
ITN	insecticide-treated net
kdr	*knockdown resistance*
nbr	number
NMCP	National Malaria Control Program
PBO	piperonyl butoxide
PCR	polymerase chain reaction
PMI	President's Malaria Initiative
PSC	pyrethrum spray catch
SS	wild type susceptible
SOP	standard operating procedure
SR	sporozoite rate
RS	heterozygous resistant
RR	homozygous resistant
VGSC	voltage gated sodium channel
WHO	World Health Organization

EXECUTIVE SUMMARY

The President's Malaria Initiative (PMI) VectorLink Project conducted entomological monitoring in the Democratic Republic of Congo (DRC) from January to December 2021. Activities took place in 12 sentinel sites distributed nationwide. The project conducted monthly longitudinal monitoring of malaria vector biting rates, resting densities, and entomological inoculation rates (EIRs) in three sites (Lodja, Kimpese, and Inongo). Indoor resting densities were determined using pyrethrum spray catch (PSC) collections, and human landing catch (HLC) was undertaken indoors and outdoors to determine malaria vector biting rates. To inform the National Malaria Control Program's (NMCP's) choice of insecticides for future insecticide-treated net (ITN) distribution campaigns, insecticide susceptibility tests were conducted in all 12 sites. Resistance intensity bioassays on *Anopheles gambiae* s.l. were conducted with permethrin, deltamethrin, and alpha-cypermethrin at one, five, and ten times the diagnostic concentration, according to World Health Organization (WHO) protocols. The project conducted synergist bioassays using piperonyl butoxide (PBO) and pyrethroids and determined susceptibility status to chlorfenapyr using the CDC bottle bioassay. The planned molecular analysis were performed at INRB between April 2021 and February 2022 All entomological data collected in 2021 was entered into VectorLink Collect and exported for data analysis. An online data visualization platform within VectorLink Collect was developed and stakeholders, including INRB, NMCP, and PMI, have access to data summaries and raw data.

Anopheles gambiae s.l. was the predominant malaria vector throughout the year in Lodja and Inongo, with *An. funestus* the predominant species collected in Kimpese. The abundance in all sites was greater from HLC (90%: 12,670/14,058) than from PSC (10%: 1,388/14,058). This indicates that *An. gambiae* s.l. and *An. funestus* s.l. Likely exit houses early in the morning (before PSC sampling which started at 6 a.m.). Biting rates of *An. gambiae* s.l. were particularly high in Lodja throughout the year and much lower in Inongo. The mean indoor resting density was less than four *An. gambiae* s.l. in Lodja, three *An. funestus* s.l. in Kimpese, and five *An. paludis* in Inongo per house per day over the reporting period. The majority of *Anopheles* collected by PSC were blood-fed in Lodja, Kimpese, and Inongo. In Lodja, the mean *An. gambiae* s.l. biting rate was 18 bites per person per night indoors and 39 outdoors, with a malaria sporozoite rate (SR) of 2.9% (70/2,400). This equates to an annual EIR of 242.56 infectious bites per person per year. In Kimpese, the mean *An. gambiae* s.l. sporozoite rate was 3.8% (32/839), with an annual EIR of 358.34 infectious bites per person. At the same site (Kimpese), *An. funestus* s.l. mean biting rate was 10 bites per person per night indoors and 11.5 bites outdoors, and a malaria sporozoite rate of 2.5% (6/240), giving an annual . EIR of 88 infectious bites per person. The combined annual EIR for both vector species was 446.34 in Kimpese. In Inongo, the mean *An. gambiae* s.l. biting rate was low (0.33 bites per person per night indoors and 0.35 bites per person per night outdoor) with no sporozoite infected mosquito.

The results highlight high year-round malaria transmission risk in Lodja and Kimpese. The data also show that there is heterogeneity across the country, with Inongo in southern DRC having a relatively low transmission risk.

Insecticide susceptibility tests showed that pyrethroid resistance is widespread. In all sites, except Lodja, *An. gambiae* s.l. were resistant to permethrin, deltamethrin and alpha-cypermethrin. Resistance intensity varied by site and by insecticide, but was usually moderate or high. Deltamethrin resistance was recorded in ten sites,

possible resistance observed in one site (Lodja) and susceptibility in one site (Katana). The intensity of deltamethrin resistance was moderate in eight sites (Inongo, Kapolowe, Karawa, Kingasani, Lisala, Mbandaka, Mweneditu, and Pawa); and high in one site (Kimpese). In most monitoring sites, resistance to the three most common pyrethroids used on ITNs (5 sites for deltamethrin 5×, 6 and 1 sites for respectively permethrin 5× and 10× and 6 and 2 sites for alpha-cypermethrin 5× and 10×, respectively) was observed at 5× and 10× concentrations, suggesting that pyrethroid ITNs are no longer providing optimal protection against malaria. The high intensity of pyrethroid resistance indicates that NMCP should consider the use of new types of ITNs for future distribution campaigns. In all sites, bioassays with permethrin following pre-exposure to PBO showed an increase in mortality compared with permethrin alone, though mortality was still <90% in nine sites. There was an increase in mortality with deltamethrin and alpha-cypermethrin following pre-exposure to PBO in all 12 sites; increases in deltamethrin mortality were particularly high (ranging from 91 to 100%) in Boende, Inongo, Kingasani, Lisala, Mbandaka, and Mweneditu but still <90% in Kapolowe, Karawa, Kimpese, and Pawa for deltamethrin and in Kapolowe, Karawa, Kimpese, Kingasani, Lisala, Mweneditu, and Pawa for alpha-cypermethrin.

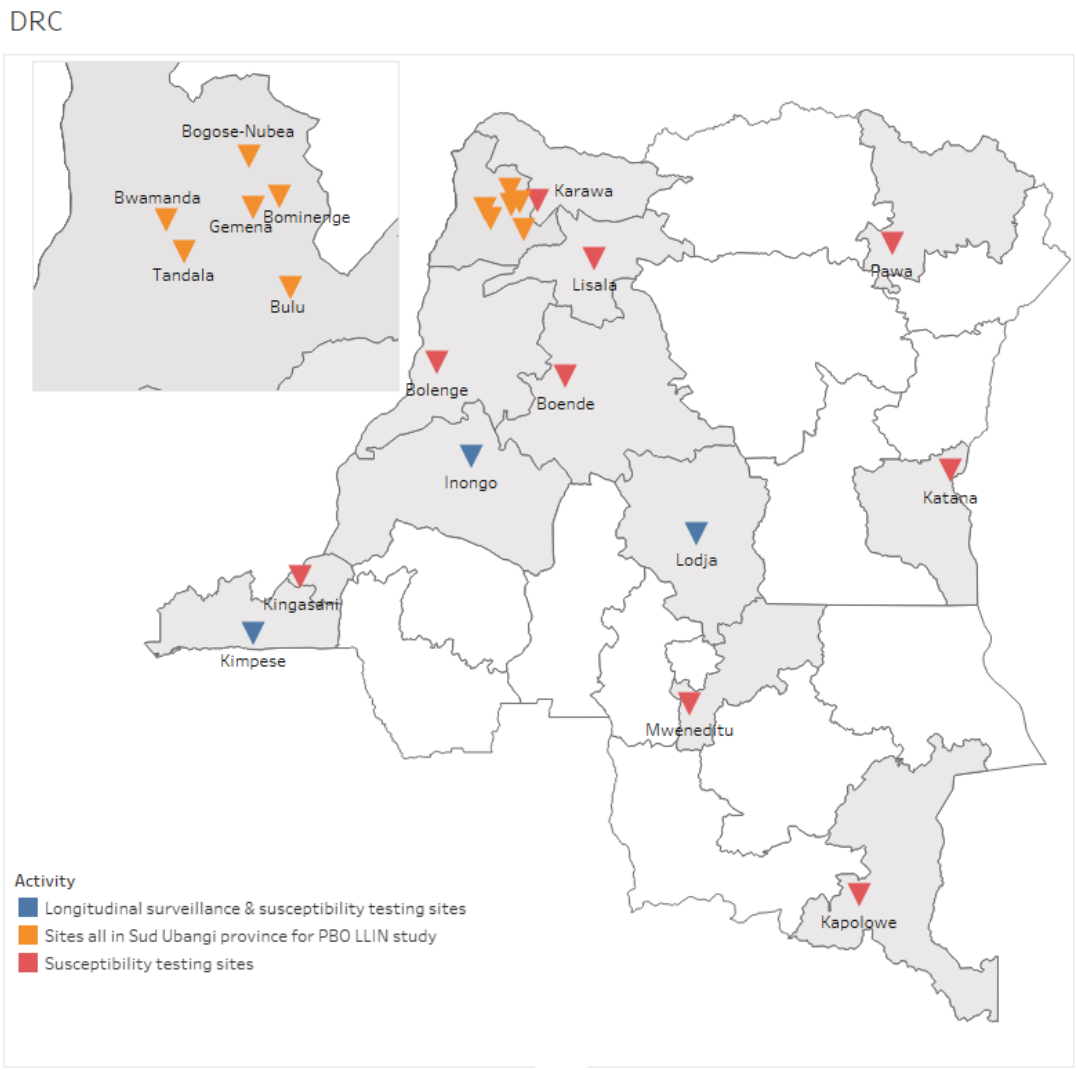
The general increase in mortality when PBO synergist was used indicates that ITNs containing PBO may provide greater control compared to pyrethroid-only ITNs, although susceptibility was not fully restored. A better option may be Interceptor G2 ITNs, as susceptibility to chlorfenapyr 100ug/bottle was recorded in seven sites (Boende, Inongo, Kimpese, Kingasani, Lisala, Mbandaka, and Mweneditu) and resistance in two sites (Karawa and Pawa) where testing was conducted.

1. METHODOLOGY

1.1 STUDY AREA

The Presidents' Malaria Initiative (PMI)'s VectorLink Project conducted entomological monitoring in 12 sites in 2021 (Figure 1). Results of a piperonyl butoxide (PBO) net study conducted in Sud Ubangi, a PBO durability study in Tanganyika, and comparisons of reported and observed insecticide treated net use in Tanganyika are presented in separate reports. Susceptibility testing that was initially scheduled in 2020 for Bolenge was re-located to Mbandaka (both in Equateur Province) due to an Ebola outbreak.

Figure 1: Location of Sentinel Sites in DRC for Entomological Monitoring in 2021.



The activities were conducted according to the PMI VectorLink DRC Year 4 work plan (Table 1).

Table 1: Summary PMI VectorLink DRC 2021 Entomological Activities.

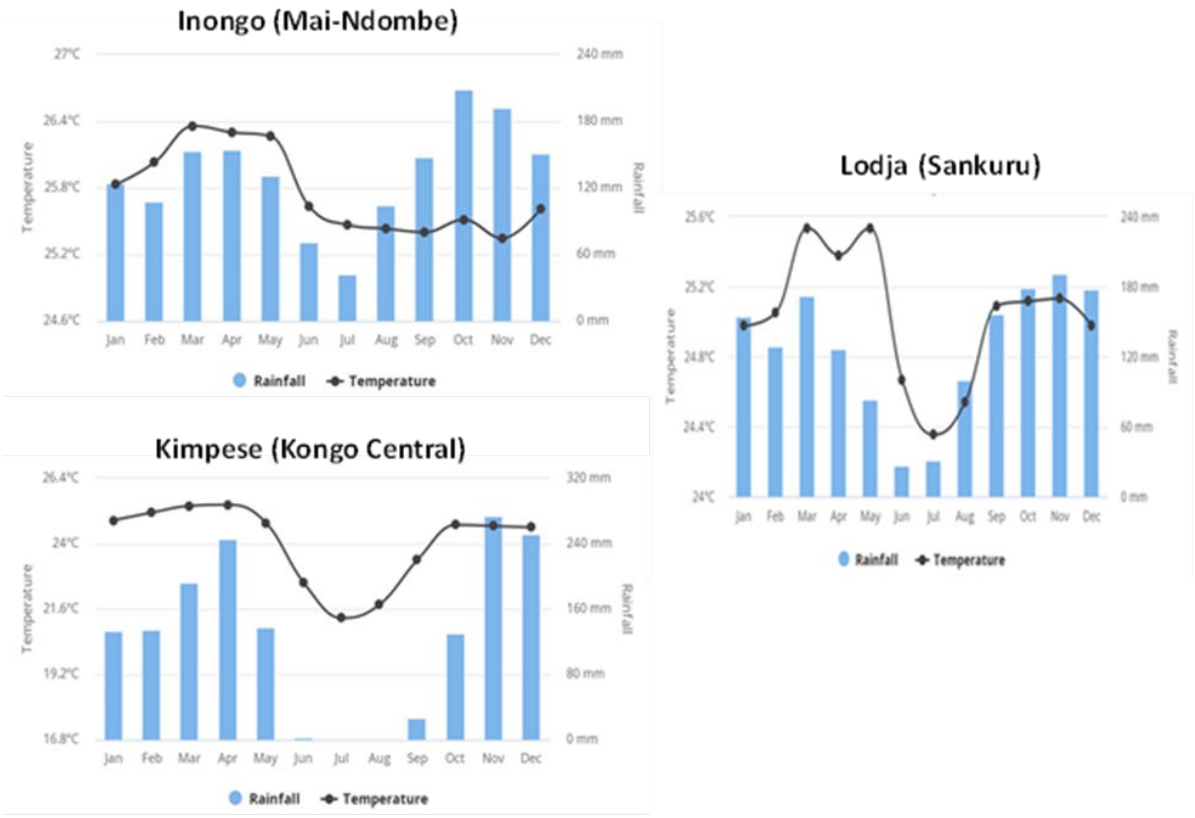
Activity	Purpose	Sites	Timeline	Frequency	Status
Vector susceptibility and intensity of resistance	• To determine vector susceptibility to three pyrethroid insecticides with/without the synergist PBO. • To determine pyrethroid resistance intensity. • To determine susceptibility to chlorfenapyr.	Boende,* Inongo, Kapolowe, Karawa, Katana, Kimpese, Kingasani, Lisala, Lodja, Mbandaka, Mweneditu, and Pawa	May–Dec 2021	12 sites, once per site	Completed
Monthly species composition, biting rate, biting times, and indoor resting densities	• To gather more detailed longitudinal information on malaria vector dynamics and behavior.	Inongo, Kimpese, and Lodja	Jan–Dec 2021	3 sites, every month	Completed
Tanganyika ITN bio-efficacy and durability	• To assess the physical and biological durability of the two ITN brands: Veeralin and SafeNet.	Kalemie and Manono	Apr–May 2021	2 sites	Completed with report approved
Comparing reported and observed ITN use in Tanganyika	• To monitor the net use and sleeping behaviors of the local populations • To compare the difference in net use by two collection methods (observed vs. reported) • To gather more information on malaria vector dynamics and behavior.	Kalemie and Manono	Apr–May and Aug 2021	2 sites, every 3 months	Completed with reports approved
Molecular assays	• To identify mosquito species of the *An. gambiae* s.l. species complex, mechanisms of pyrethroid resistance (*kdr*), and sporozoite rates.	Boende,* Inongo, Kapolowe, Karawa, Katana, Kimpese, Kingasani, Lisala, Lodja, Mbandaka, Mweneditu, and Pawa	Jan–Dec 2021	12 sites	Completed

Activity	Purpose	Sites	Timeline	Frequency	Status
Sud Ubangi ITN monitoring	• To collect data on the density of vectors and the susceptibility of *An. gambiae* s.l. to deltamethrin with and without PBO • To conduct ITN bioassay testing of PBO and pyrethroid nets using locally collected *An. gambiae* s.l. from Gemena.	Sud Ubangi in 6 health zones	Jan–Feb and Aug–Sep 2021	6 sites, once per site	Completed for 6- and 12-months with reports approved

Note: ITN=insecticide-treated net, *kdr=knockdown resistance*, PBO=piperonyl butoxide, PCR=polymerase chain reaction.
*New site for 2021.

Figure 2 shows the mean rainfall and temperature for the three longitudinal monitoring sites (Inongo, Lodja, and Kimpese). All three have a dry season in the middle of the year, which lasts approximately four months in Lodja and Inongo and six months in Kimpese. Inongo has considerable rainfall for most of the year, with peaks in March/April and October/November. The mean temperature in Inongo is quite stable year-round, with a mean of 25–26°C, providing perfect conditions for malaria vector survival.

Figure 2: Average Monthly Temperature and Rainfall (1991–2016) in Longitudinal Entomological Monitoring Sites.



Source: Climatic Research Unit of University of East Anglia

Mosquitoes were collected monthly using human landing catch (HLC) and pyrethrum spray catch (PSC) in Lodja, Inongo, and Kimpese. See Table 2 for a summary of collection methods. Houses were sampled monthly in the same village each month in the district of Inongo (Maman Yaka: −1.92249, 18.30027), in the health zone of Asani (−3.53636, 23.58333), and in the district of Cataractes Yanga Diansonga (−5.57166, 14.42333).

Table 2: Summary of Collection Methods.

Collection Method	Time	Frequency	Sample
Human landing catch	6:00 p.m. to 6:00 a.m.	4 days per site per month	8 houses per site per month (2 houses per night)
Pyrethrum spray catch	6:00 a.m. to 9:00 a.m.	2 days per site per month	10 ouses per site per month (5 houses per day)

1.2 Human Landing Catch

HLCs were performed to assess mosquito biting time, feeding behavior, and biting rates and to monitor species composition and sporozoite rates. Trained residents collected adult mosquitoes over four consecutive nights in two different houses each night (total of eight houses per month), with one person located indoors and another outdoors in each selected house. Collectors rotated indoors and outdoors every hour. The same houses were used every month in Lodja, Kimpese and Inongo. All *Anopheles* mosquitoes collected by HLC per the standard operating procedure (SOP) 02/01,[1] were identified to species morphologically in the field and cross-checked by National Institute of Biomedical Research (INRB) entomologists either in the field or in Kinshasa (depending on the supervision schedule). All *Anopheles* were preserved in 1.5 ml Eppendorf tubes on silica gel for further molecular analysis in Kinshasa by INRB.

1.3 Pyrethrum Spray Catch

PSCs were conducted following the SOP 03/01[2] from 6:00 a.m. to 9:00 a.m. in the same areas as for HLC (but in different houses) to estimate the indoor resting density of mosquito species. Before the PSCs were performed, all occupants were asked to move out of the house. The rooms were sprayed with a commercially available aerosol containing pyrethroid and PBO to knock down mosquitoes resting inside the house. Twenty minutes after spraying, all mosquitoes knocked down were collected from a white sheet lying on the flat surfaces. Female *Anopheles* were classified according to the four abdominal stages (unfed, fed, half-gravid, and gravid). Each mosquito collected was labeled properly, stored in an Eppendorf tube with silica gel, identified to species morphologically in the field, and cross-checked by an INRB entomologist once back at the central lab.

[1] 2-SOP Human landing catch. PMI VectorLink Entomology Standard Operating Procedures: https://pmivectorlink.org/resources/tools-and-innovations/

[2] 3-SOP Pyrethrum spray catch. PMI VectorLink Entomology Standard Operating Procedures: https://pmivectorlink.org/resources/tools-and-innovations/

1.4 Insecticide Susceptibility, PBO Synergist, and Resistance Intensity Testing

Insecticide susceptibility and resistance intensity testing were conducted per the SOP 06/01[3] in 12 sentinel sites. As part of decentralization of entomological surveillance, insecticide susceptibility testing was conducted by the staff from three of the sentinel sites, with no supervision from INRB. The three sites were Katana, Kapolowe and Lodja, where local staff are relatively well trained. Insecticide papers were sent from Kinshasa to the remote sites; after testing, mosquito samples and data sheets were returned to INRB. As chlorfenapyr testing is more challenging technically and requires controlled test conditions, it was not undertaken in these sites. Susceptibility testing was conducted in another nine sites (Boende, Inongo, Karawa, Kimpese, Kingasani, Lisala, Mbandaka, Mweneditu, and Pawa) with in-person supervision from INRB. Of the 12 sites, Boende was sampled for the first time.

In addition to tests at the diagnostic dose, World Health Organization (WHO) intensity bioassays were also conducted by testing with pyrethroid papers treated with five and 10 times the diagnostic dose. The insecticides tested in 2021 were:

- Deltamethrin ×1, ×5, ×10 (0.05%, 0.25%, 0.5%)
- Permethrin ×1, ×5, ×10 (0.75%, 3.75%, 7.5%)
- Alpha-cypermethrin ×1, ×5, ×10 (0.05%, 0.25%, 0.5%)
- Deltamethrin 0.05% with pre-exposure to PBO 4%
- Permethrin 0.75% with pre-exposure to PBO 4%
- Alpha-cypermethrin 0.05% with pre-exposure to PBO 4%
- Chlorfenapyr 100µg/bottle[4]

In all sites, susceptibility testing was conducted with adult *An. gambiae* s.l. following the WHO method (with the exception of chlorfenapyr).

WHO susceptibility tests were conducted on deltamethrin, permethrin, and alpha-cypermethrin, with pre-exposure to PBO for 60 minutes[3] to determine the change in mortality rates with PBO exposure.

CDC bottle bioassays were completed following the SOP 04/01[5] in the nine sites (Boende, Inongo, Karawa, Kimpese, Kingasani, Lisala, Mbandaka, Mweneditu, and Pawa) to determine the susceptibility status of *An. gambiae* s.l. populations to chlorfenapyr using an interim diagnostic dose of 100µg/bottle. Four replicates of 20-25 *An. gambiae* s.l. were exposed for 60 minutes to chlorfenapyr 100ug/bottle.

[3] 6-SOP WHO Susceptibility test. PMI VectorLink Entomology Standard Operating Procedures: https://pmivectorlink.org/resources/tools-and-innovations/

[4] Chlorfenapyr was tested following the Centers for Disease Control and Prevention bottle bioassays.

[5] 4-SOP CDC Bottle bioassay. PMI VectorLink Entomology Standard Operating Procedures: https://pmivectorlink.org/resources/tools-and-innovations/

1.5 MOLECULAR ANALYSIS

Molecular analyses were conducted in a new molecular laboratory at INRB, which was equipped by the project and dedicated to entomology. The mosquito samples collected from sentinel sites were transported to INRB for processing and analysis. Technicians conducted laboratory analyses under the supervision of the INRB focal point entomologist and PMI VectorLink entomologist, following the protocols described in Table 3.

Table 3: Protocols Used for Laboratory Analysis of Malaria Vectors.

Molecular Analysis	Protocol	Output
ELISA	Malaria Research and Reference Reagent Resource Centre document "*Anopheles* in Research" (based on Wirtz et al. [1987])	Sporozoite identification: Identified mosquito samples that were positive for *Plasmodium falciparum* sporozoites
PCR	Santolamazza et al. (2008)	Species identification: Identified *An. gambiae* complex sibling species including *An. coluzzii*, *An. gambiae,* and *An. arabiensis*
	Huynh et al. (2007)	Vgsc-L1014F allele frequency: Monitored pyrethroid target site resistance mechanism frequency

Note: ELISA=enzyme-linked immunosorbent assay, PCR=polymerase chain reaction.

Sporozoite infection rate testing was completed for 3,304 *An. gambiae* s.l. collected from Kimpese, Lodja, and Inongo in 2021 using an enzyme-linked immunosorbent assay (ELISA) and 2,427 *An. gambiae* s.l. using polymerase chain reaction (PCR) for species identification and resistance mechanism detection (see detail in Table 4). The results are reported below in Sections 2.6 and 2.8. ELISA tests were conducted on a subsample of *Anopheles* collected through HLCs in Lodja, Inongo, and Kimpese (targeted at a sample of 200 *An. gambiae* s.l. per month, or 2,400 total and 20 *An. funestus* s.l. per month, or 240 total). PCR analysis was performed on a subsample of *An. gambiae* s.l. collected through HLCs and susceptibility tests in 12 sites. The number of *An. gambiae* s.l. analyzed by each method in each sentinel site is shown in Table 4.

Table 4: Number of *An. gambiae* s.l. Samples Analyzed at INRB, Kinshasa, Compared with Number Planned

Sentinel Site	Total *An. gambiae* s.l. Collected in 2021 by HLC	HLC Sporozoite ELISA Monthly Monitoring**	HLC Species ID Monthly Monitoring**	WHO Pyrethroid Susceptibility Test, Species ID**	WHO Pyrethroid Susceptibility Test, VGSC L1014F frequency**
Lodja	5,416	2,400/2,400	600/600	100/100	50/50
Kimpese*	839	839/2,400	600/600	100/100	50/50
Inongo*	65	65/2,400	27/600	100/100	50/50
Kapolowe	-	-	-	100/100	50/50
Katana	-	-	-	100/100	50/50
Kingasani	-	-	-	100/100	50/50
Karawa	-	-	-	100/100	50/50
Pawa	-	-	-	100/100	50/50
Mbandaka	-	-	-	100/100	50/50

Sentinel Site	Total *An. gambiae* s.l. Collected in 2021 by HLC	HLC Sporozoite ELISA Monthly Monitoring**	HLC Species ID Monthly Monitoring**	WHO Pyrethroid Susceptibility Test, Species ID**	WHO Pyrethroid Susceptibility Test, VGSC L1014F frequency**
Mweneditu	-	-	-	100/100	50/50
Lisala	-	-	-	100/100	50/50
Boende	-	-	-	100/100	50/50
Total	**6,320**	**3,304/7,200**	**1,227/1,800**	**1,200/1,200**	**600/650**

*Only 65 *An. gambiae* s.l. were collected in Inongo and 839 in Kimpese (January–December 2021).
**Numbers represent the total number tested in the reporting period/the total number targeted. For those where the targets were not met, not enough mosquitoes could be found in those sites.

1.6 Data Management and Analysis

The DHIS2-based VectorLink Collect programs for entomological data management were used. The Home Office staff remotely supported INRB and Abt entomologist technicians and database managers on updated data work flows – including field paper collections, technical reviews, data entry, data cleaning, and analytics – to support the generation and use of high-quality entomological data. All entomological data collected in DRC in 2021 was managed within VectorLink Collect. The platform includes comprehensive dashboards to synthesize vector bionomics and insecticide resistance summary results. Stakeholders, including INRB, NMCP, and PMI, have access to these results dashboards to support timely decision-making.

The following formulas were used to calculate entomological indicators:

- Sporozoite rate = (total ELISA positive/total number tested) × 100
- Human biting rate (HBR) = total # of each *Anopheles* species collected by HLCs during a specific period/total number of trap nights
- Nightly EIR = nightly HBR × sporozoite rate
- Monthly EIR = nightly mean EIR × number of nights in the month
- Yearly EIR = addition of monthly EIRs

The WHO recommendations below are used for interpretation of results from susceptibility and the intensity bioassays:

- Susceptible when a mortality in the range of 98-100% is recorded at the diagnostic dose
- Resistance when a mortality <90% is recorded at the diagnostic dose
- Possible resistance when a mortality of 90-97% is recorded at the diagnostic dose
- Low resistance intensity when a mortality of 98-100% is recorded at the 5× concentration
- Moderate resistance intensity when a mortality <98% is recorded at the 5× concentration
- High resistance intensity when a mortality of <98% is recorded at the 10× concentration

2. RESULTS

2.1 MALARIA VECTOR SPECIES COMPOSITION

Over the study period (January to December 2021), a total of 14,058 *Anopheles* mosquitoes were collected from the three longitudinal monitoring sites through monthly HLC and PSC. Six *Anopheles* species (*An. gambiae* s.l., *An. funestus* s.l., *An. paludis, An. coustani, An. tenebrosus,* and *An. nili*) were collected, with *An. gambiae* s.l. being the most common (*n*=6,866), followed by *An. paludis* (*n*=4,640), and *An. funestus* s.l. (*n*=2,404).

In all sites, the abundance of *Anopheles* species was greater from HLC (90%: 12,670/14,058) than from PSC (10%: 1,388/14,058). *Anopheles paludis* was the predominant species in Inongo, but was not found in Kimpese. The species composition is presented by site and collection method in Figures 3, 4, and 5. Details about the indoor resting densities and biting rates are presented in Sections 2.2 and 2.3. Molecular species composition is presented in Section 2.8.

Figure 3: Species Composition of *Anopheles* Captured by PSC and HLC (Indoors and Outdoors) in Kimpese from Monthly Collections

Fig. 3a: Kimpese, PSC-Indoor, Jan–Dec 2021 (*n*=344)

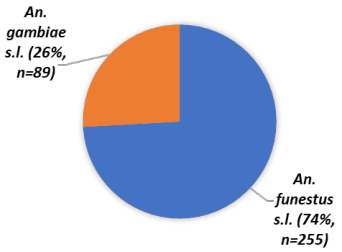


Fig. 3b: Kimpese, HLC-Indoor, Jan–Dec 2021 (*n*=1,403)

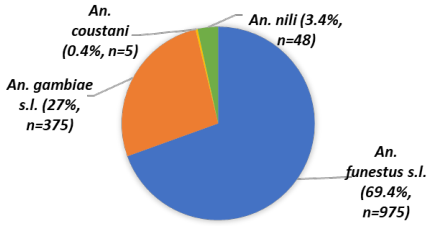


Fig. 3c: Kimpese, HLC-outdoor, Jan–Dec 2021 (*n*=1,624)

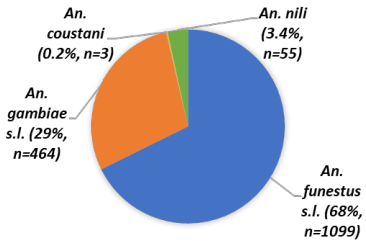


Figure 4: Species Composition of *Anopheles* Captured by PSC and HLC (Indoors and Outdoors) in Lodja from Monthly Collections

Fig 4a: Lodja, PSC-Indoor, Jan–Dec 2021 (*n*=469)

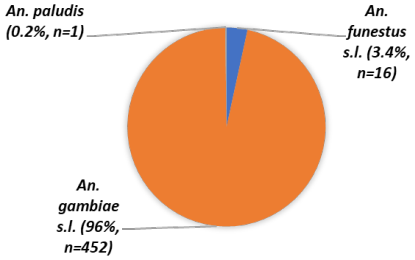


Fig. 4b: Lodja, HLC-Indoor, Jan–Dec 2021 (*n*=2,059)

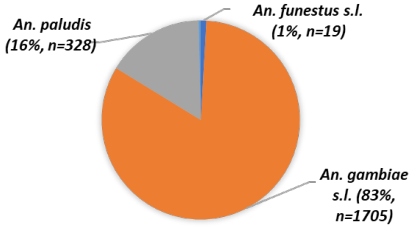


Fig. 4c: Lodja, HLC-outdoor, Jan–Dec 2021 (*n*=5,039)

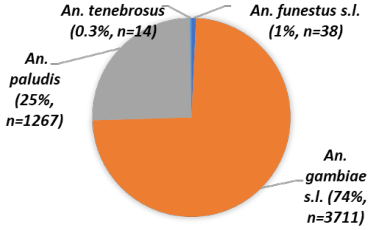


Figure 5: Species Composition of *Anopheles* Captured by PSC and HLC (Indoors and Outdoors) in Inongo from Monthly Collections

Fig.5a: Inongo, PSC-Indoor, Jan–Dec 2021 (*n*=575)

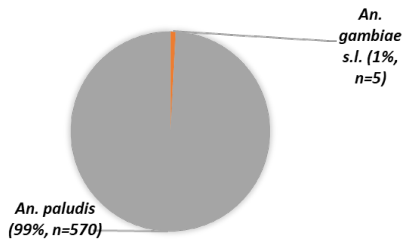


Fig. 5b: Inongo, HLC-Indoor, Jan–Dec 2021 (*n*=1,330)

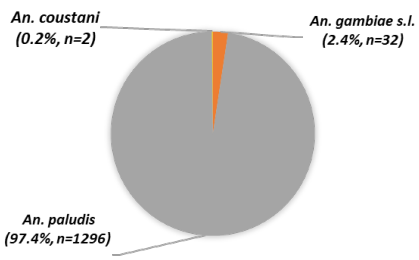
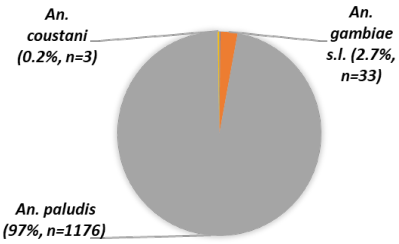


Fig. 5c: Inongo, HLC-outdoor, Jan–Dec 2021 (*n*=1,215)



2.2 Malaria Indoor Vector Resting Density (by PSC)

Figures 6, 7, and 8 show the mean indoor resting density of *An. gambiae* s.l., *An. funestus* s.l., and *An. paludis* per house per day collected by PSC. In Lodja, the indoor resting density was fairly stable throughout the year, with a peak of *An. gambiae* s.l. in December. The mean indoor resting density per house per day was less than four *An. gambiae* s.l. in Lodja, three *An. funestus* s.l. in Kimpese, and five *An. paludis* in Inongo. The majority of *Anopheles* mosquitoes collected were blood-fed (47% overall) in Lodja, Kimpese, and Inongo.

Figure 6: Mean Indoor Resting Density per House per Day of *An. gambiae* s.l. Captured by PSC in Lodja (*n*=452), Kimpese (*n*=89), and Inongo (*n*=5) from Monthly Collections

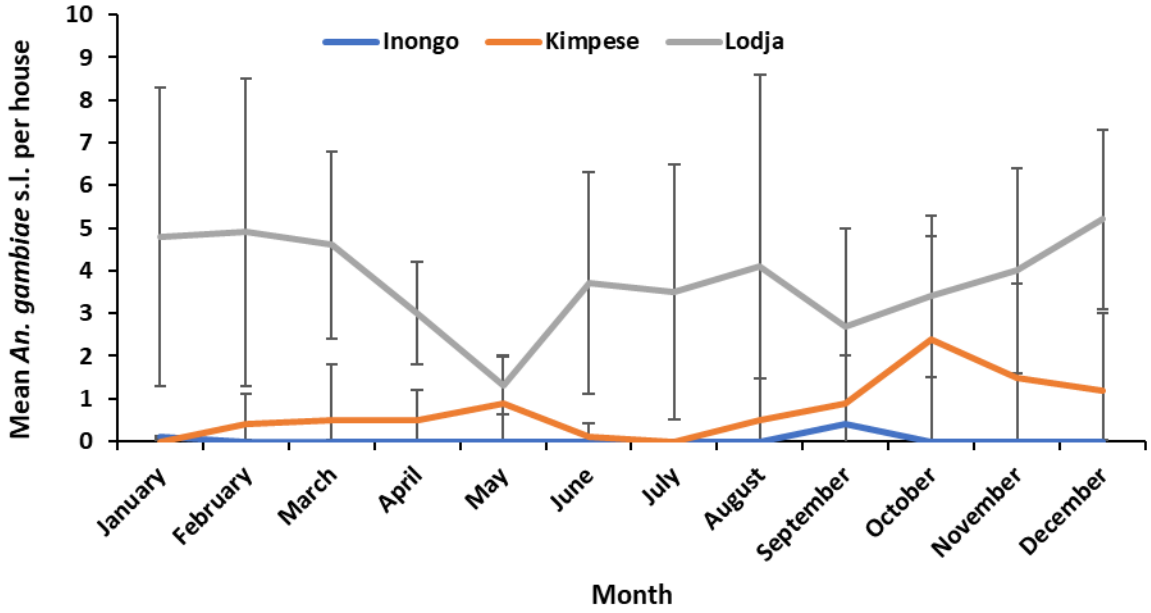


Figure 7: Mean Indoor Resting Density per House per Day of *An. funestus* s.l. Captured by PSC in Kimpese (*n*=255) and Lodja (*n*=16) from Monthly Collections

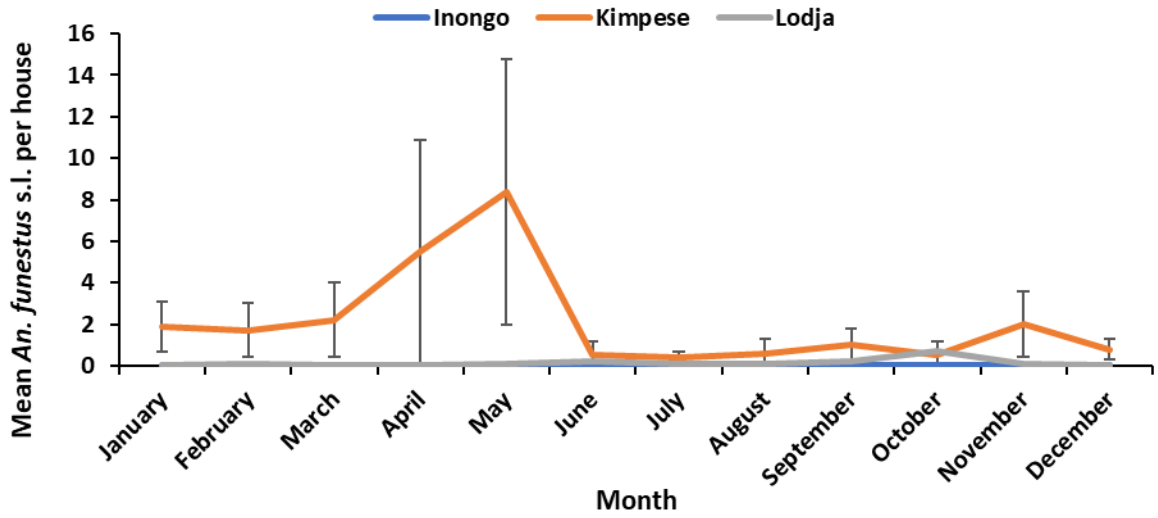
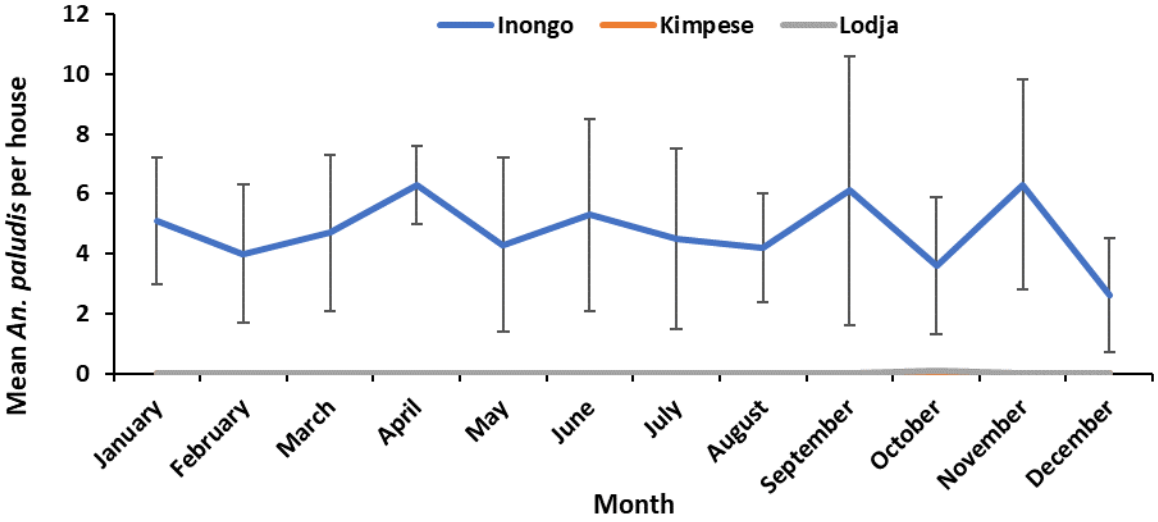


Figure 8: Mean Indoor Resting Density per House per Day of *An. paludis* Captured by PSC in Inongo (*n*=570) and Lodja (*n*=1) from Monthly Collections



2.3 Monthly Malaria Vector Human Biting Rates (by HLC)

Figures 9 to 14 show the mean monthly biting rates per person per night in Lodja, Kimpese, and Inongo by vector species (details in Annex, Tables 10, 11, and 12).

Anopheles gambiae s.l. biting rates were particularly high in Lodja, with a mean of 18 bites per person per night indoors and 39 outdoors. The biting rates were consistent year-round in Lodja, with high rates observed in the rainy season between January to May. Biting rates of An. gambiae s.l. in Inong was very low year-round with a mean biting rate of 0.3 bites per person per night indoors and 0.4 bites outdoors (Figures 9 and 10).

Biting was seasonal in Kimpese, with most biting occurring between November and March.

Figure 9: Mean Monthly Indoor *An. gambiae* s.l. Biting Rate in Lodja (*n*=1,705), Kimpese (*n*=375), and Inongo (*n*=32)

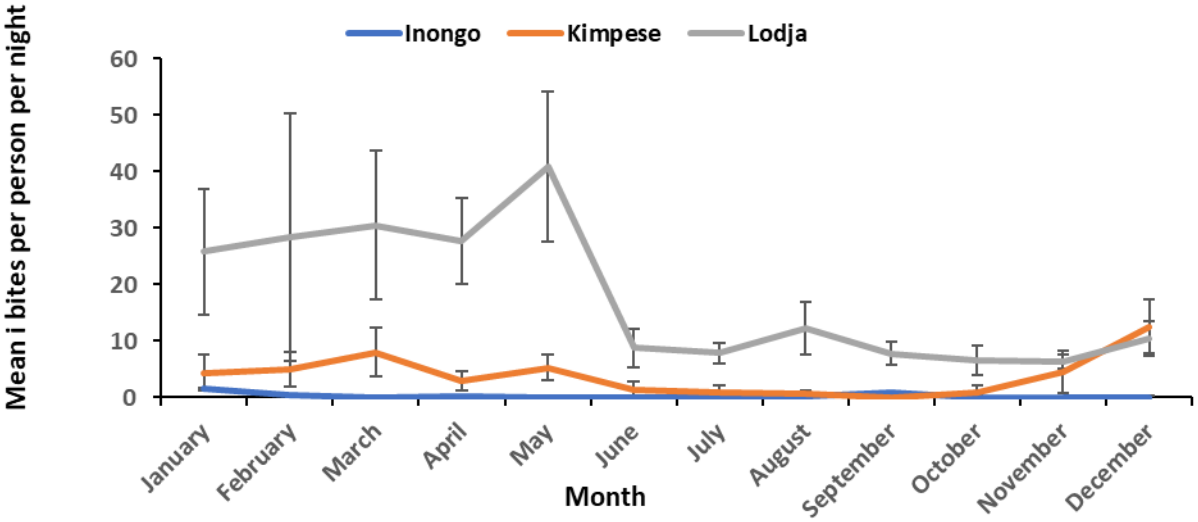
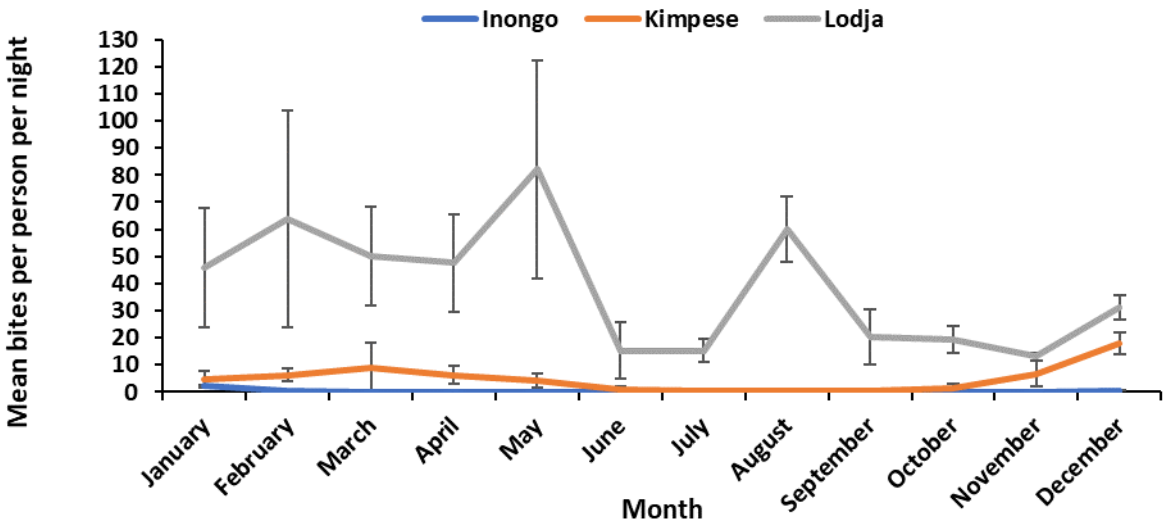


Figure 10: Mean Monthly Outdoor *An. gambiae* s.l. Biting Rate in Lodja (*n*=3,711), Kimpese (*n*=464), and Inongo (*n*=33)



An. funestus s.l. was most commonly collected in Kimpese (>20 mean bites per person per night), with peak biting between December and January during the rainy season and between April and June in the dry season (Figures 11 and 12).

Figure 11: Mean Monthly Indoor *An. funestus* s.l. Biting Rate in Lodja (*n*=19), Kimpese (*n*=975), and Inongo (*n*=0)

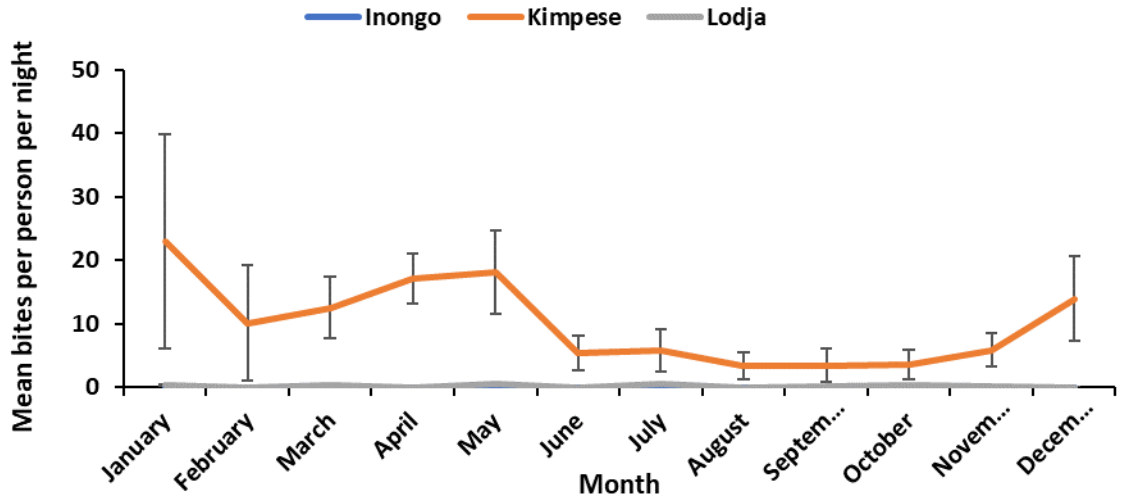
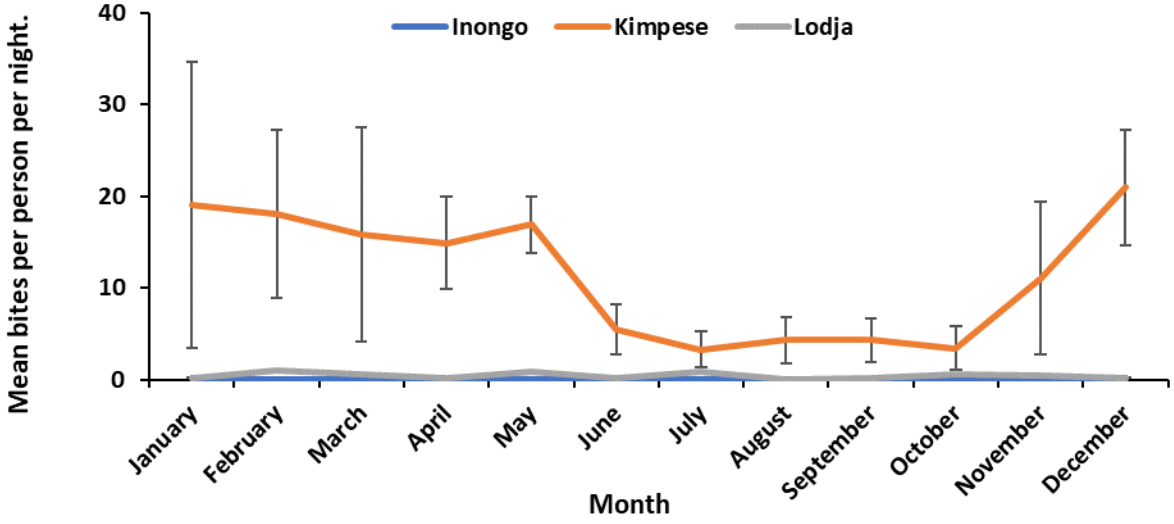


Figure 12: Mean Monthly Outdoor *An. funestus* s.l. Biting Rate in Lodja (*n*=38), Kimpese (*n*=1,099), and Inongo (*n*=2)



The most common species collected in Inongo was *An. paludis*, which is unlikely to be an important malaria vector species (Figures 13 and 14).

Figure 13: Mean Monthly Indoor *An. paludis* Biting Rate in Lodja (*n*=328), Kimpese (*n*=0), and Inongo (*n*=1,296)

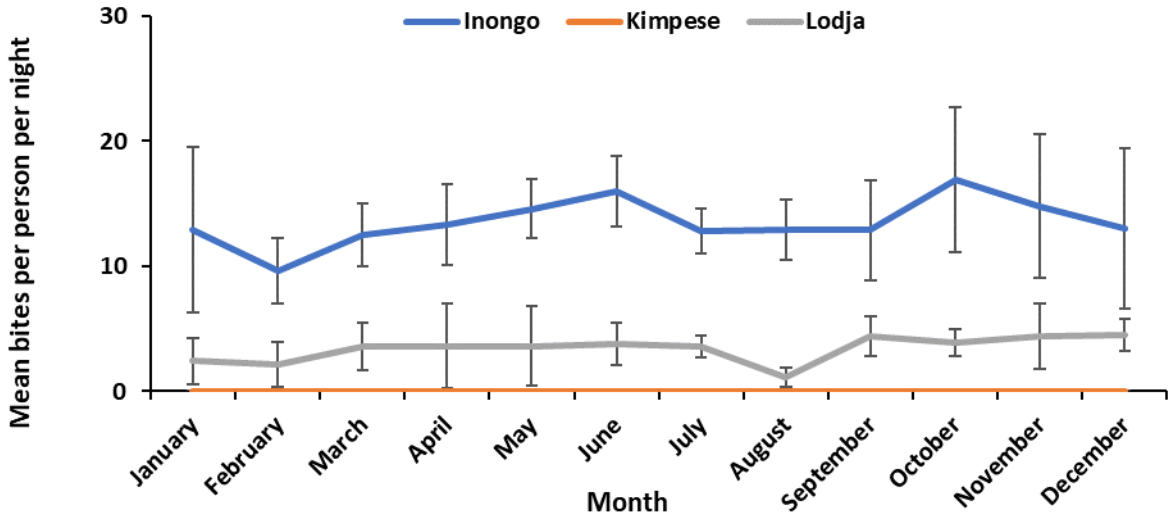
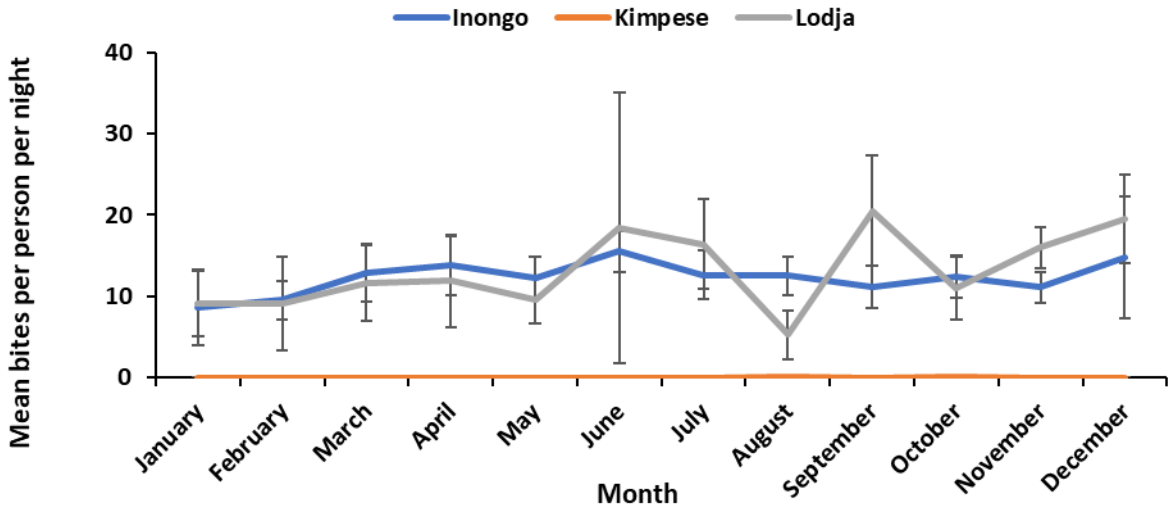


Figure 14: Mean Monthly Outdoor *An. paludis* Biting Rate in Lodja (*n*=1,267), Kimpese (*n*=2), and Inongo (*n*=1,176)



2.4 Biting Times of Malaria Vectors Collected Indoors and Outdoors by HLC

In general, the peak period of *An. gambiae* s.l. and *An. funestus* s.l. indoor biting was late at night, between 10 p.m. and 5 a.m., which mirrored outdoor biting trends in almost all sites (Figures 15 to 20). Biting rates in Lodja

were substantially greater outdoors than indoors, particularly between 9 p.m. and 2 a.m. The peak period of *An. paludis* indoor biting was earlier, between 6 p.m. and 11 p.m., which mirrored outdoor biting trends in Inongo and Lodja (Figures 19 and 20).

Figure 15: Mean Hourly Indoor *An. gambiae* s.l. Biting Rates in Lodja (*n*=1,705), Kimpese (*n*=375), and Inongo (*n*=32)

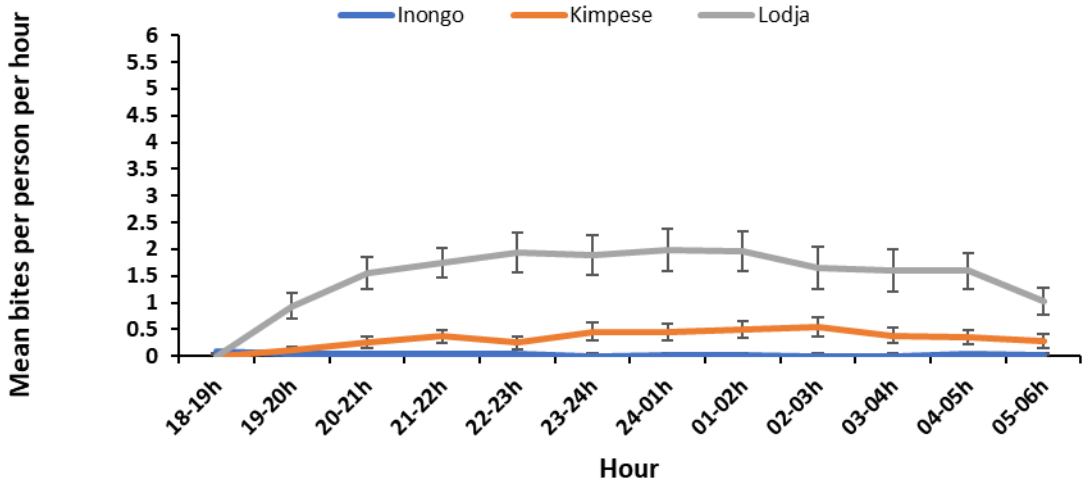


Figure 16: Mean Hourly Outdoor *An. gambiae* s.l. Biting Rates in Lodja (*n*=3,711), Kimpese (*n*=464), and Inongo (*n*=33)

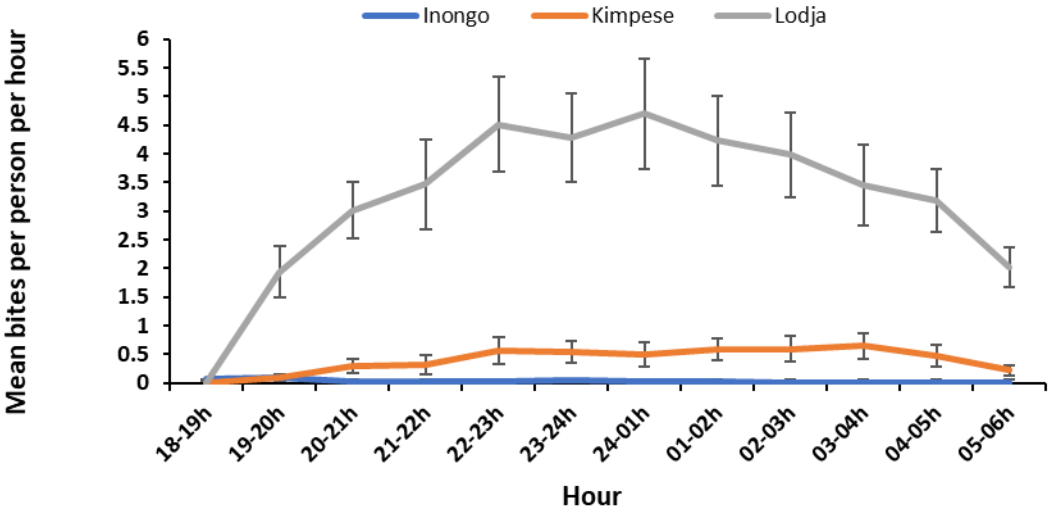


Figure 17: Mean Hourly Indoor *An. funestus* s.l. Biting Rates in Lodja (*n*=19), Kimpese (*n*=975), and Inongo (*n*=0)

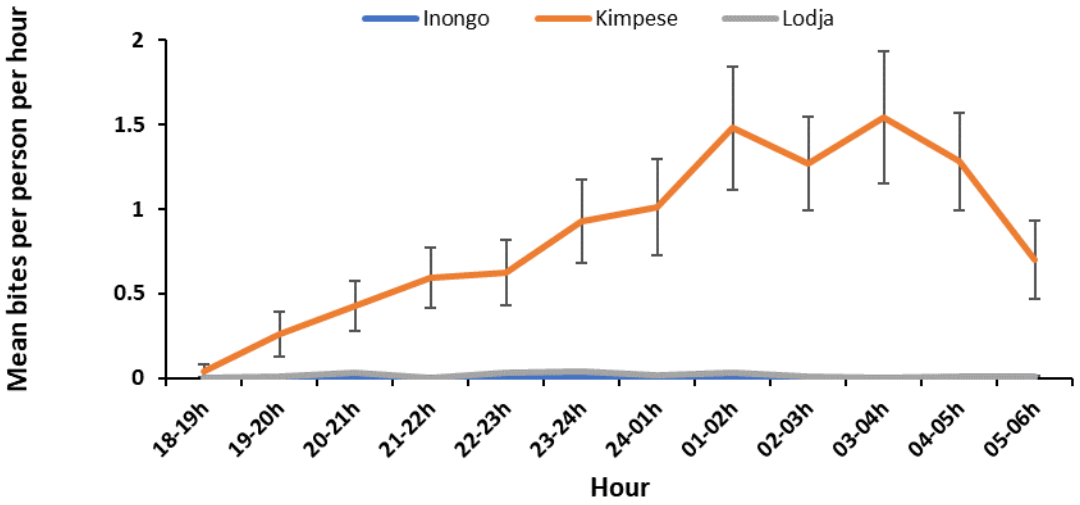


Figure 18: Mean Hourly Outdoor *An. funestus* s.l. Biting Rates in Lodja (*n*=38), Kimpese (*n*=1,099), and Inongo (*n*=2)

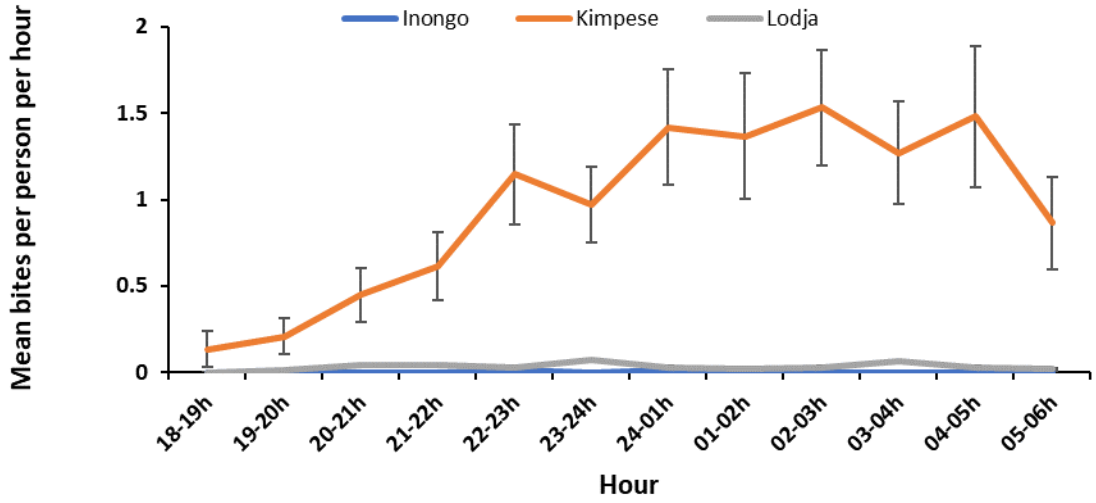


Figure 19: Mean Hourly Indoor *An. paludis* Biting Rates in Lodja (*n*=328), Kimpese (*n*=0), and Inongo (*n*=1,296)

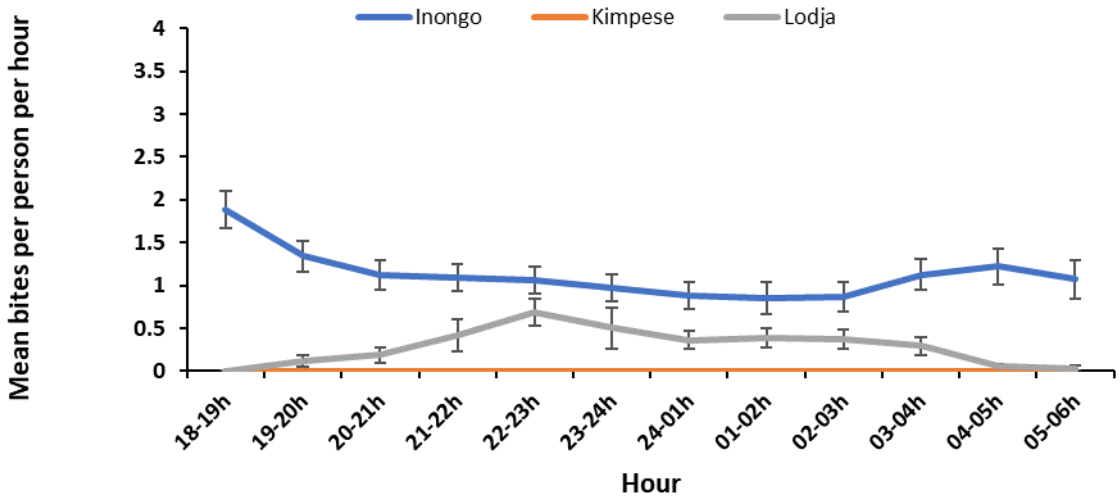
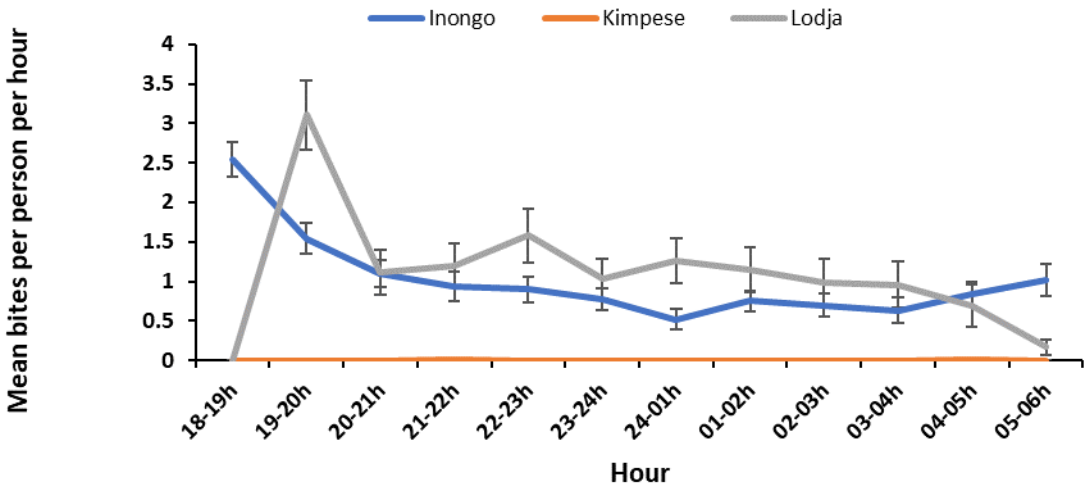


Figure 20: Mean Hourly Outdoor *An. paludis* Biting Rates in Lodja (*n*=1,267), Kimpese (*n*=2), and Inongo (*n*=1176)



2.5 *PLASMODIUM FALCIPARUM* SPOROZOITE RATE

The number of *An. gambiae* s.l. and *An. funestus* s.l. analyzed for presence of sporozoites from each site is shown in Table 5. All work plan targets for processing mosquitoes were met, except for Kimpese and Inongo due to an insufficient number of *An. gambiae* s.l. specimens collected through HLC (all 839 and 65 *An. gambiae* s.l. collected respectively from Kimpese and Inongo were analyzed in the laboratory).

Table 5: Number of *An. gambiae* s.l. and *An. funestus* s.l. Samples Collected by HLC and Tested for Presence of *P. falciparum* Sporozoites

Sentinel Site	Total *An. gambiae* s.l. Collected by HLC	Total *An. gambiae* s.l. Tested (tested/planned)	% Mean Sporozoite Rate (positive/ tested)	Total *An. funestus* s.l. Collected by HLC in 2020	Total *An. funestus* s.l. Tested	% Sporozoite Rate (positive/ tested)
Kimpese	839**	839/2,400 (all collected)	3.8% (32/839)	2,074	240 (20 per month)	2.5% (6/240)
Lodja	5,416	2,400/2,400 (200 per month)	2.9% (70/2,400)	57*	0	n/a
Inongo	65**	65/2,400 (all collected)	0% (0/65)	2*	0	n/a
Total	**6,320**	**3,304**	**3.1% (102/3,304)**	**2,133**	**240**	**2.5% (6/240)**

Note:
*Insufficient *An. funestus* s.l. captured in Lodja and Inongo for testing.
** Insufficient *An. gambiae* s.l. captured in Kimpese and Inongo.

The mean *An. gambiae* s.l. infection rate over 12 months in Lodja was 2.9% (95% confidence interval (CI), 2.32–3.67); 3.8% in Kimpese (95% CI, 2.71–5.33); and 0% in Inongo. The mean *An. funestus* s.l. sporozoite rate in Kimpese was 2.5% (95% CI, 1.15–5.35). In Lodja and Inongo, no *An. funestus* were tested due to low numbers collected (Table 5). The monthly *An. gambiae* s.l. sporozoite rate for Lodja and Kimpese from HLCs is presented in Figures 21 and 22. There is no figure presented for Inongo due to the low number of *An. gambiae* s.l. collected and 0% sporozoite rate recorded.

Although there appeared to be peaks of infection (e.g., Lodja in March and November), the confidence intervals were quite large, as only 200 mosquitoes were tested per month and it was not possible to clearly determine any seasonality in infection rate. Because *An. funestus* s.l. was the main species collected in Kimpese, it would be beneficial to increase the number of samples tested for sporozoites to determine the monthly infection rate and more accurately determine the overall EIR for 2021. These additional tests will be conducted using 2021 samples during Year 5 with a view to future publication of the longitudinal data.

Figure 21: Monthly *P. falciparum* Sporozoite Rate of *An. gambiae* s.l. Collected by HLC in Lodja

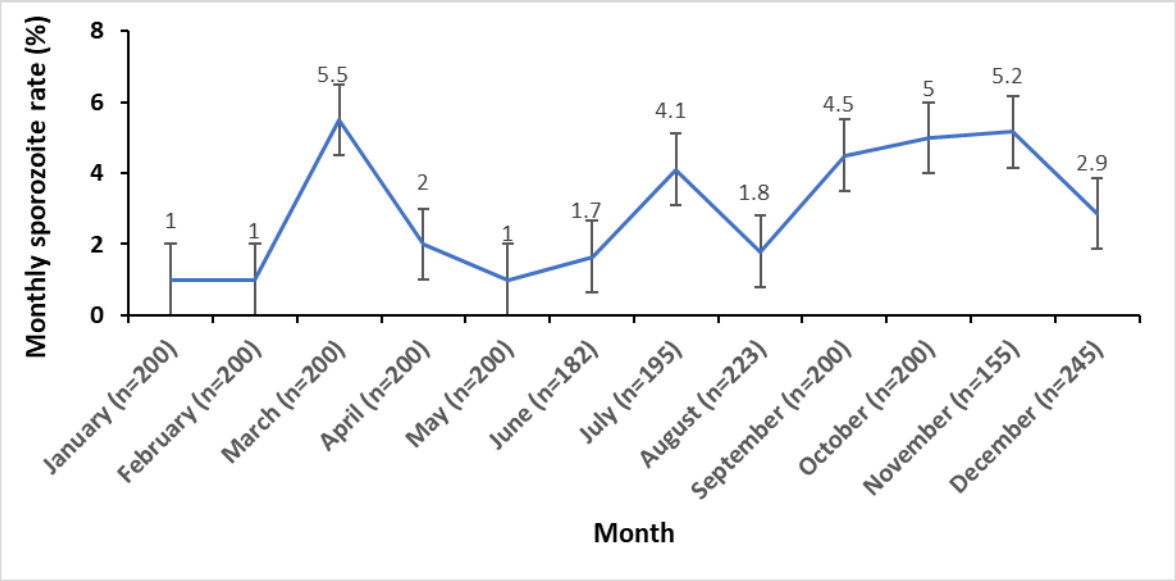
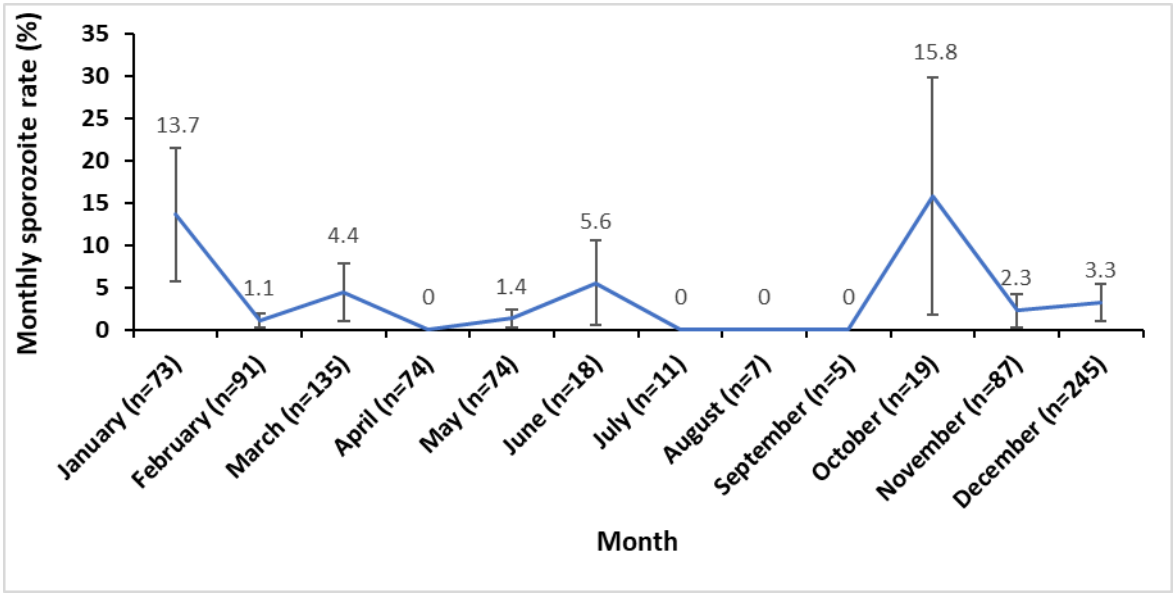


Figure 22: Monthly *P. falciparum* sporozoite Rate of *An. gambiae* s.l. Collected by HLC in Kimpese



2.6 Entomological Inoculation Rate

Tables 6 and 7 summarize the combined indoor and outdoor monthly EIRs for *An. gambiae* s.l. in Lodja and Kimpese for 2021. Addition of the monthly EIR gave an annual EIR of 242.56 infectious bites per person per year for Lodja and 358.34 for Kimpese. No sporozoite-positive *An. gambiae* s.l. were detected in Inongo in 2021, giving an annual EIR of 0 infectious bites per person per year.

Sporozoite-positive *An. funestus* s.l. were detected in Kimpese only in January, April, July, August, and November, giving an estimated annual EIR of 88 infectious bites per person:

The combined annual EIR (*An. funestus* s.l. and *An. gambiae* s.l.) in Kimpese was 446.34 infectious bites per person per year. Overall, these results demonstrate the high malaria transmission risk faced year-round in Lodja and Kimpese, despite the use of pyrethroid insecticide-treated nets (ITNs). However, the malaria transmission risk was relatively low in Inongo.

Table 6: Monthly *An. gambiae* s.l. EIR in Lodja

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	2021 Total
Total *An. gambiae* s.l. (HLC) collected	572	737	644	603	984	192	185	579	224	207	155	334	**5,416**
HLC trap nights (indoors + outdoors)	16	16	16	16	16	16	16	16	16	16	16	16	**192**
HBR per night	36	46	40	38	62	12	12	36	14	13	10	21	**28 (mean)**
Total *An. gambiae* s.l. tested by ELISA	200	200	200	200	200	182	195	223	200	200	155	245	**2,400**
Sporozoite rate (%)	1	1	5.5	2	1	1.7	4.1	1.8	4.5	5	5.2	2.9	**2.9 (mean)**
EIR per night	0.36	0.46	2.20	0.76	0.62	0.20	0.49	0.65	0.63	0.65	0.1	0.61	**0.81 (mean)**
EIR per month*	11.16	12.88	68.2	28.8	19.22	6	15.19	20.15	18.9	20.15	3	18.91	**20.21 (mean)**
Lodja 12 month EIR Jan–Dec 2021 = 242.56 infectious bites per person (by *An. gambiae* s.l.)													

Note: EIR=entomological inoculation rate, ELISA=enzyme-linked immunosorbent assay, HBR=human bite rate, HLC=human landing catch.
*Nightly EIR is multiplied by number of nights in that month.

Table 7: Monthly *An. gambiae* s.l. EIR in Kimpese

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	2021 Total
Total *An. gambiae* s.l. (HLC) collected	72	90	136	74	74	18	11	7	5	18	90	244	**839**
HLC trap nights (indoors + outdoors)	16	16	16	16	16	16	16	16	16	16	16	16	**192**
HBR per night	35.75	46.06	40.25	37.69	61.5	12	11.56	36.19	14	12.94	9.69	20.88	**4.37 (mean)**
Total *An. gambiae* s.l. tested by ELISA	73	91	135	74	74	18	11	7	5	19	87	245	**839**
Sporozoite rate (%)	13.7	1.1	4.4	0	1.4	5.6	0	0	0	15.8	2.3	3.3	**3.81 (mean)**
EIR per night	4.90	0.51	1.79	0	0.83	0.67	0	0	0	2.04	0.22	0.68	**0.17 (mean)**
EIR per month*	151.82	14.17	55.46	0	25.76	20	0	0	0	63.33	6.68	21.13	**29.86 (mean)**
Kimpese 12 month EIR Jan–Dec 2021 = 358.34 infectious bites per person (by *An. gambiae* s.l.)													

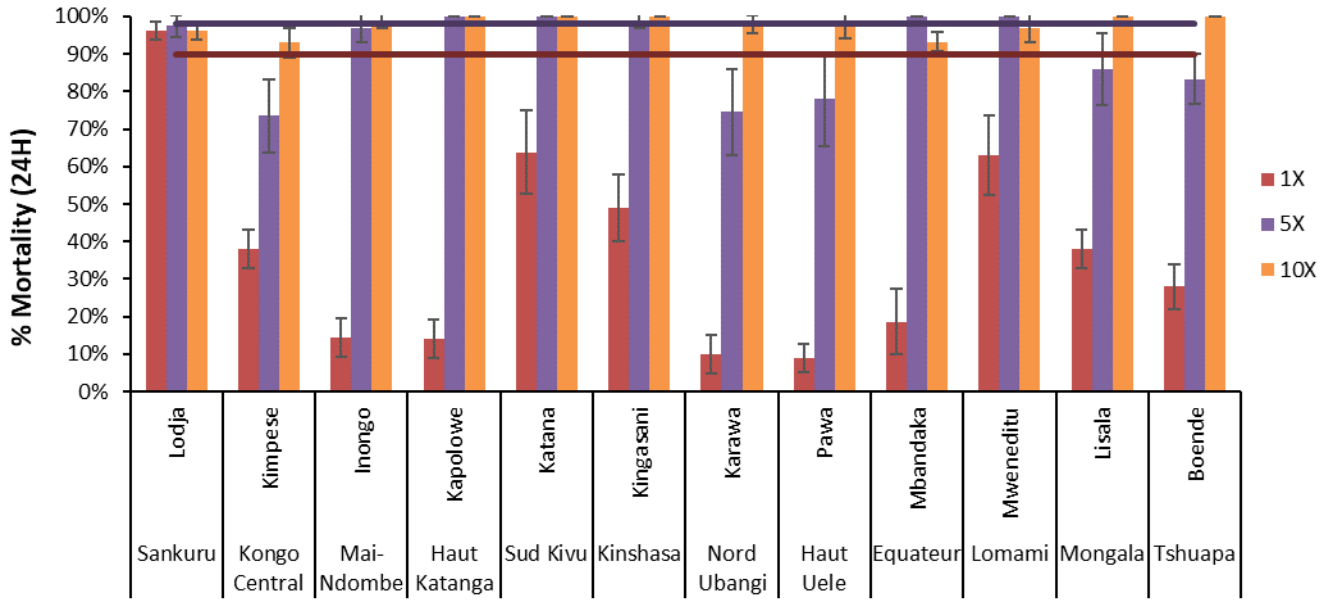
***Nightly EIR is multiplied by number of nights in that month.

2.7 Insecticide Susceptibility, PBO Synergist, and Pyrethroid Resistance Intensity

WHO insecticide susceptibility and resistance intensity tests were completed with *An. gambiae* s.l. populations that were collected as larvae in all 12 sites. While following the PMI COVID-19 mitigation plan, provincial technicians in the sites with no INRB supervision (Katana, Kapolowe, and Lodja) were able to conduct insecticide susceptibility testing (including PBO synergist tests) with success.

Figure 23 shows the percentage mortality of Anopheles gambiae s.l. in x1, x5 and x10 doses of permethrin , Resistance to permethrin (×1 dose) observed in all sites except Lodja, where the *An. gambiae* s.l. population tested showed possible resistance (to be confirmed in 2022). Resistance intensity to permethrin was low (>98% mortality) in five sites (Kapolowe, Katana, Kingasani, Mbandaka, and Mweneditu); moderate (<98% mortality) in five sites (Inongo, Karawa, Pawa, Lisala, and Boende); and high (<98% mortality at ×10 dose) in one site (Kimpese).

Figure 23: Percentage Mortality of *An. gambiae* s.l. after Exposure to Permethrin at ×1, ×5, and ×10 the Diagnostic Concentration in WHO Tube Tests in 12 Sites.



As shown in Figure 24, resistance to deltamethrin was recorded in 10 sites (×1 dose). Possible resistance was observed in one site (Lodja) and susceptibility in one site (Katana). The intensity of deltamethrin resistance was moderate in eight sites (Inongo, Kapolowe, Kingasani, Karawa, Pawa, Mbandaka, Mweneditu, and Lisala) and high in one site (Kimpese).

Resistance to alpha-cypermethrin was also observed in 11 sites (Figure 25) and possible resistance observed in one site (Lodja). The intensity of alpha-cypermethrin resistance was moderate in six sites (Lodja, Inongo, Karawa, Pawa, Mweneditu, and Lisala) and high in four sites (Kimpese, Kapolowe, Kingasani, and Mbandaka) and low in two sites (Katana and Boende).

Figure 24: Percentage Mortality of *An. gambiae* s.l. after Exposure to Deltamethrin at ×1, ×5, and ×10 the Diagnostic Concentration in WHO Tube Tests in 12 Sites

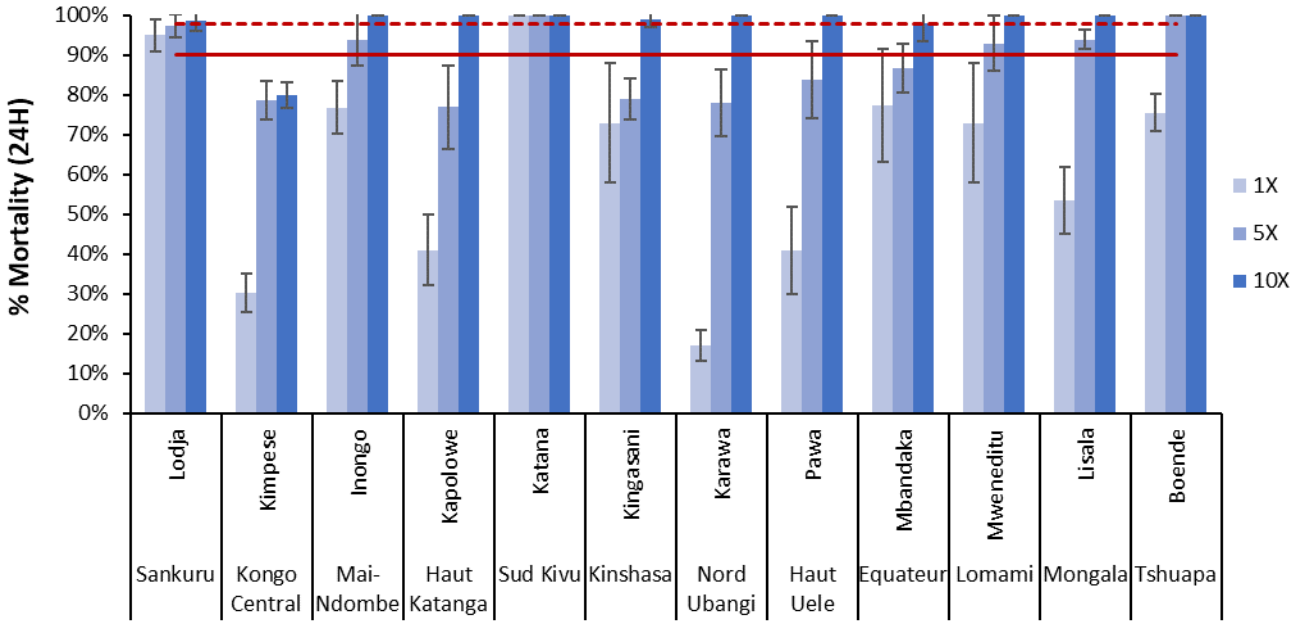
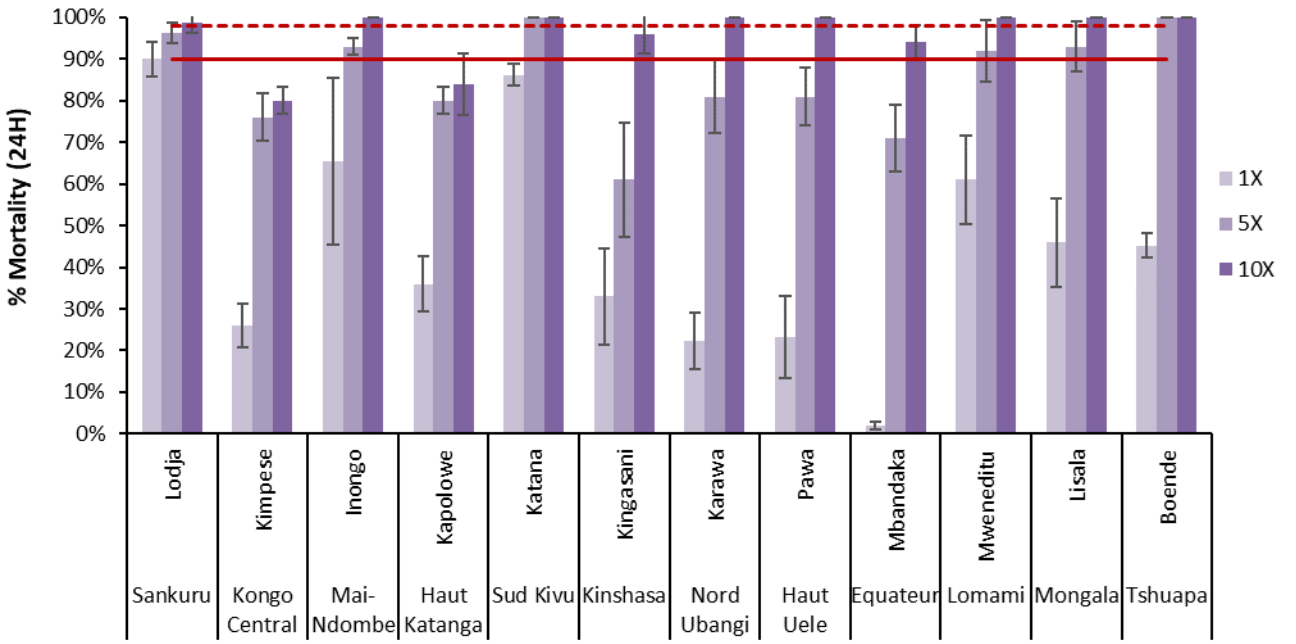
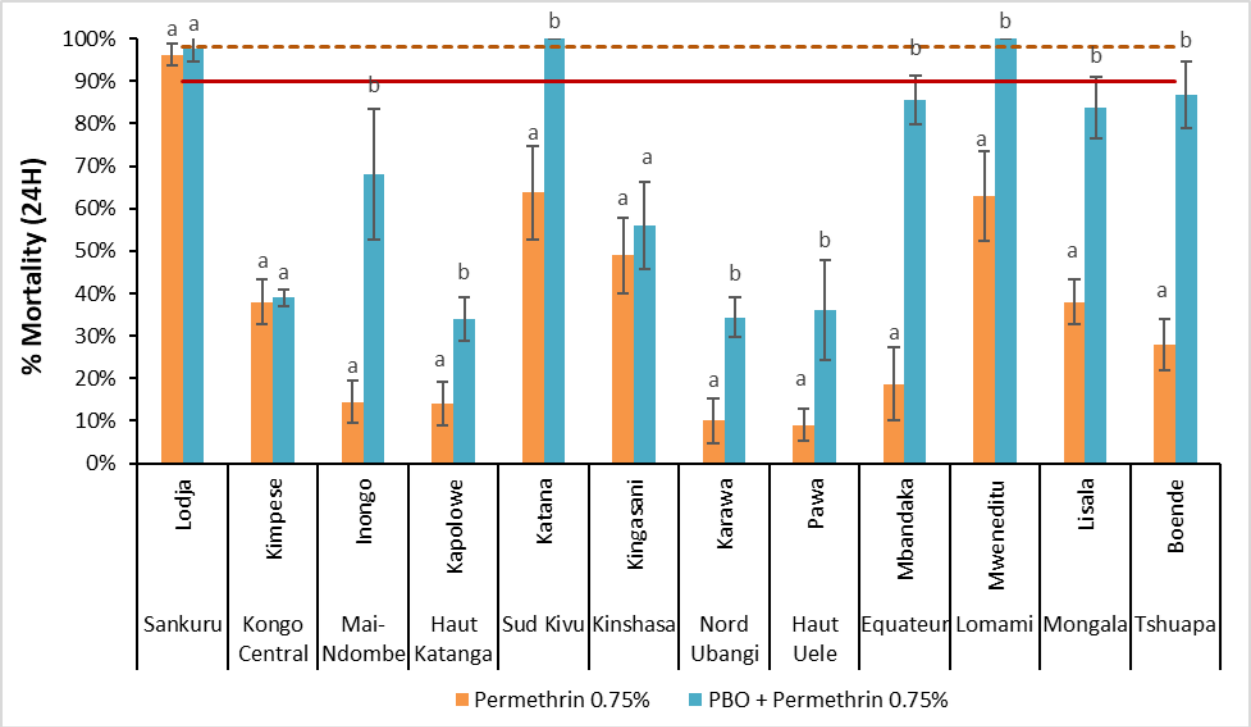


Figure 25: Percentage Mortality of *An. gambiae* s.l. after Exposure to Alpha-Cypermethrin at ×1, ×5, and ×10 the Diagnostic Concentration in WHO Tube Tests in 12 Sites



Bioassays with permethrin (×1 dose) following pre-exposure to PBO 4% in WHO tube tests showed an increase in mortality ranged from 34-100% compared with permethrin alone in all sites (Figure 26). There was no significant increase in mortality in Kimpese and Kingasani. Despite an increase in mortality after pre-exposure to PBO, mortality was still <90% in nine sites (Kimpese, Inongo, Kapolowe, Kingasani, Karawa, Pawa, Mbandaka, Lisala and Boende).

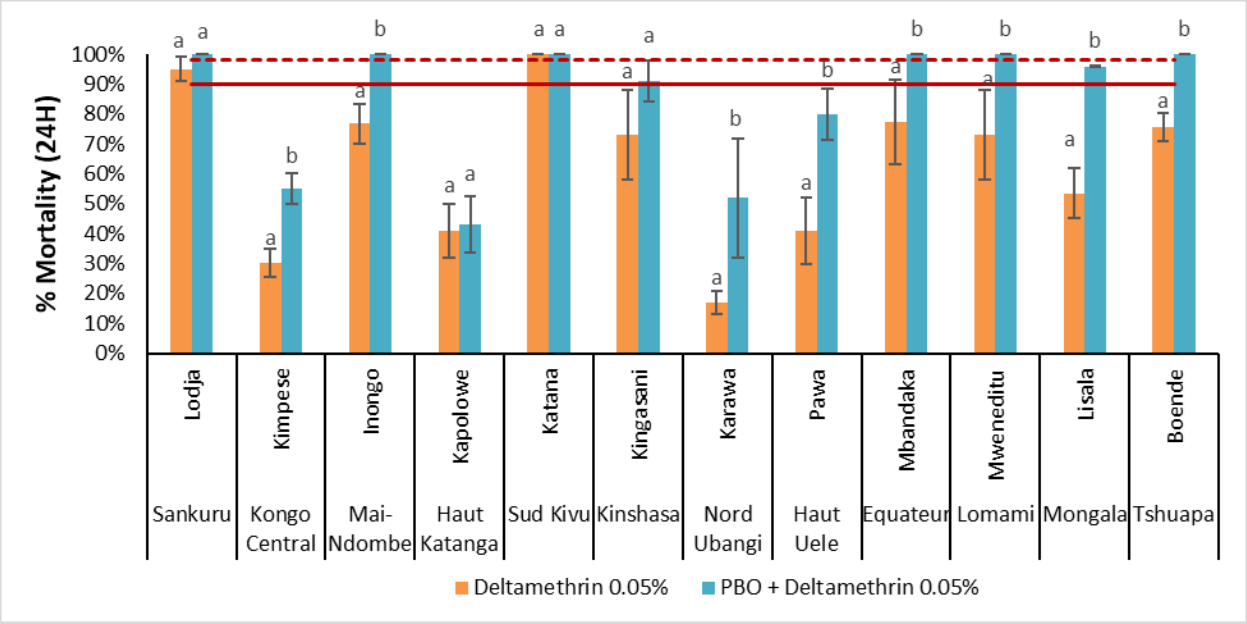
Figure 26: Percentage Mortality of *An. gambiae* s.l. after Pre-Exposure to PBO Followed by Permethrin at the Diagnostic Concentration in WHO Tube Tests in 12 Sites



Note: Superscripts indicate whether % mortality for permethrin is significantly different from % mortality for permethrin + PBO.
a, b = significant difference, p<.05.
a, a = no significant difference, p>.05.

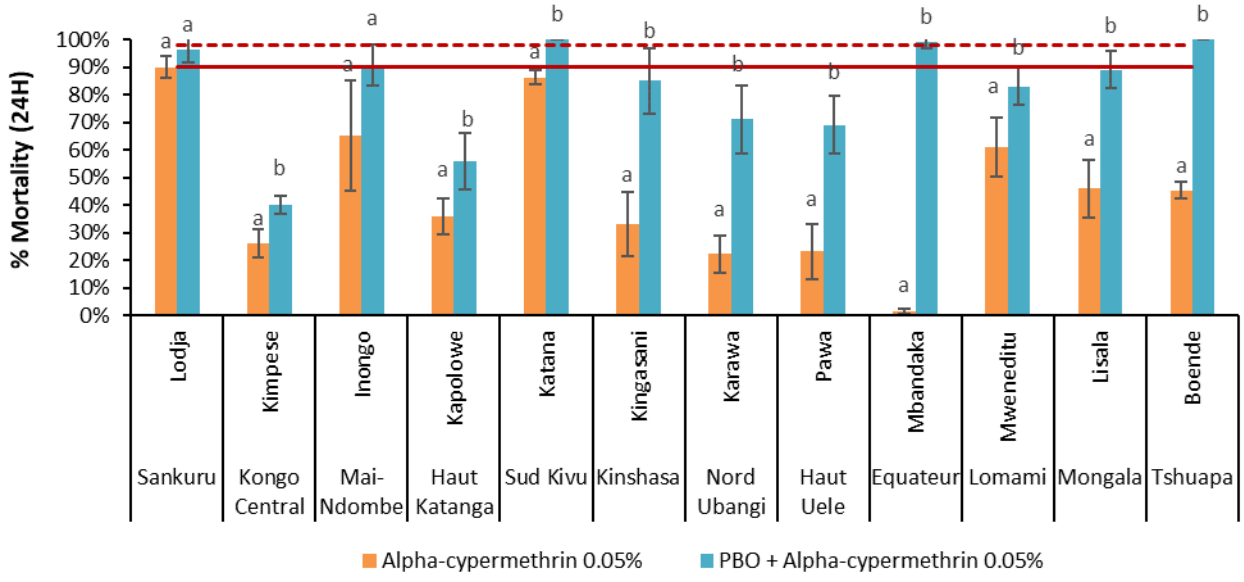
There was a significant increase in mortality with deltamethrin and alpha-cypermethrin (×1 dose) following pre-exposure to PBO 4% in WHO tube tests in nine and 10 sites, respectively (Figures 27 and 28), except Kapolowe for PBO + alpha-cypermethrin. For deltamethrin, there were significant increases in mortality (>90% mortality, P < 0.05%) in Inongo, Mbandaka, Mweneditu, Lisala, and Boende and unsignificant increase in Kingasani (Figure 27). Despite an increase in mortality after pre-exposure to PBO, mortality was still <90% in Kimpese, Kapolowe, Karawa, and Pawa for deltamethrin and in Kimpese, Kapolowe, Kingasani, Karawa, Pawa, Mweneditu, and Lisala for alpha-cypermethrin (Figures 27 and 28).

Figure 27: Percentage Mortality of *An. gambiae* s.l. after Pre-Exposure to PBO Followed by Deltamethrin at the Diagnostic Concentration in WHO Tube Tests in 12 Sites



Note: Superscripts indicate whether % mortality for deltamethrin is significantly different to % mortality for deltamethrin + PBO.
a, b = significant difference, $p<.05$.
a, a = no significant difference, $p>.05$.

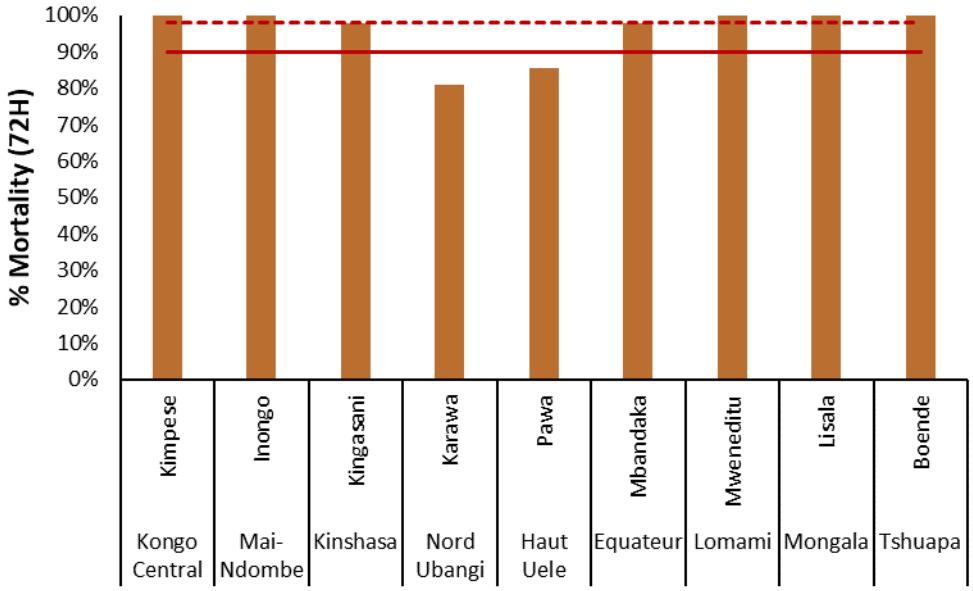
Figure 28: Percentage Mortality of *An. gambiae* s.l. after Pre-Exposure to PBO Followed by Alpha-cypermethrin at the Diagnostic Concentration in WHO Tube Tests in 12 Sites



Note: Superscripts indicate whether % mortality for alpha-cypermethrin is significantly different from % mortality for alpha-cypermethrin + PBO.
a, b = significant difference, $p<.05$.
a, a = no significant difference, $p>.05$.

CDC bottle bioassays using 100µg/bottle as the diagnostic dose for chlorfenapyr produced 100% mortality in five sites (Kimpese, Inongo, Mweneditu, Lisala, and Boende), >90% mortality in two sites (Kingasani and Mbandaka), and <90% mortality in two sites (Karawa and Pawa) within 72 hours of exposure (Figure 29). Testing with 200 µg/bottle should be performed when mortality < 98% is recorded after 72 hours of exposure.

Figure 29: Percentage Mortality of *An. gambiae* s.l. after Exposure to Chlorfenapyr 100ug/bottle in CDC Bottle Tests in Nine Sites



2.8 MOLECULAR SPECIES IDENTIFICATION OF THE *AN. GAMBIAE* SPECIES COMPLEX

Out of 2,427 *An. gambiae* s.l. analyzed, 90% were identified as *An. gambiae* . (*n*=2,171), 7% as *An. coluzzii* (*n*=81), and 0.04% as *An. arabiensis* (*n*=1). Seven percent (*n*=174) did not amplify. No hybrid (*An. gambiae* s.s./*An. coluzzii*) was detected (Figure 30).

Figure 30: Molecular Species Composition of *An. gambiae* across 12 Sites

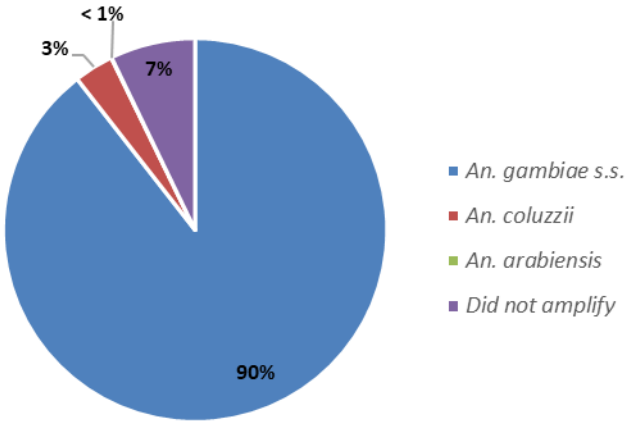


Table 8 shows the proportion of each species per site (collected by HLC), and Figure 31 shows molecular species composition of those mosquitoes analyzed through WHO susceptibility tests. In Inongo, 100% of *An. gambiae* s.s. were collected. In Lodja and Kimpese, there were sympatric populations of *An. gambiae* and *An. coluzzii*, with *An. gambiae* the most common species collected in both sites (84% and 96% of amplified samples, respectively). Samples from monthly HLC collections in Lodja, Kimpese, and Inongo showed no clear seasonal variation in species composition. *An. arabiensis* was detected at very low frequency (1%) in Katana from *An. gambiae* s.l. collected as larvae for WHO susceptibility tests (Figure 30).

Table 8: Molecular Species Composition of *An. gambiae* s.l. Collected by HLC

Site	*An. gambiae* s.s.	*An. coluzzii*	Hybrid	*An. arabiensis*	Did Not Amplify	2021 Total
Lodja	501 **(84%)**	74 **(12%)**	0	0	25 **(4%)**	**600**
Kimpese	578 **(96%)**	1 **(0.2%)**	0	0	21 **(3.5%)**	**600**
Inongo	27 **(100%)**	0	0	0	0	**27**

Figure 31: Molecular Species Composition of *An. gambiae* s.l. Collected as Larvae for WHO Susceptibility Tests

An. gambiae was the predominant species used in WHO susceptibility tests (collected as larvae) at all 12 sites. *An. coluzzii* were detected in Kingasani (6%) (Figure 31).

2.9 Frequency of the Voltage Gated Sodium Channel Mutation L1014F

From a total of 600 *An. gambiae* and *An. coluzzii* specimens analyzed, 522 (87%) were homozygous for Vgsc-L1014F, 37 (6%) were wild type susceptible, and 8 (1%) heterozygous resistant. The remaining 33 (6%) did not amplify (Table 9). The high proportion of homozygous resistant samples is in support previous results of *An. gambiae* s.l. collected from 11 sites in 2018 that were tested by CDC, Atlanta, and found to be 85% (883/1,039) homozygous resistant compared to only 3% wild type susceptible or heterozygous resistant (28/1,039), with the remainder not amplified.[6]

[6] Wat'senga et al. (2020). Intensity of pyrethroid resistance in *Anopheles gambiae* before and after a mass distribution of insecticide-treated nets in Kinshasa and in 11 provinces of the Democratic Republic of Congo. *Malaria Journal*, 19; 169.

Table 9: Frequency of the Vgsc L1014F Mutation (Formely *kdr*-west) in *An. gambiae* s.s. and *An. coluzzii*

Site	Number Tested	Homozygous Resistant	Heterozygous Resistant	Wild Type Susceptible	Did Not Amplify	Frequency 1014F
Lodja	50	49	0	0	1	**1.0**
Kimpese	50	47	0	0	3	**1.0**
Inongo	50	46	0	0	4	**1.0**
Kapolowe	50	47	0	0	3	**1.0**
Katana	50	47	0	0	3	**1.0**
Kingasani	50	47	0	0	3	**1.0**
Karawa	50	49	0	0	1	**1.0**
Pawa	50	25	15	8	2	**0.68**
Mbandaka	50	42	5	0	3	**0.95**
Mweneditu	50	47	0	0	3	**1.0**
Lisala	50	32	17	0	1	**0.83**
Boende	50	44	0	0	6	**1.0**

3. CAPACITY BUILDING

VectorLink worked to strengthen capacity at the central and provincial level. Dr. Rodrigue Agossa, the in-country entomologist, supported project activities by assisting with data entry, cleaning and analysis in VectorLink Collect, updating and translating SOPs for susceptibility and intensity tests, WHO cone bioassays, Prokopack aspiration, molecular species identification, and *kdr* mutation detection into French. Mr. Agossa worked with INRB and the University of Kinshasa to implement activities. He also provided technical assistance to NMCP to support vector control working group activities and NMCP's training in applied entomology. Other efforts are summarized below.

Training	• VectorLink M&E conducted a remote training in November for INRB and VectorLink DRC staff on the new functionality of VectorLink Collect. All 2021 entomological data (HLC, PSC, and susceptibility data) were successfully entered and cleaned in VectorLink Collect. • VectorLink, through INRB, identified and trained 3 supervisors (3 per site) in the new Boende sites. The training consisted of basic bio-ecology of *An. gambiae* s.l., adult mosquito sampling methodology, and WHO susceptibility tests. • The VectorLink Chief of Party facilitated sessions on resistance and bio-ecology of vector modules as part of an NMCP applied entomology training, funded by Global Fund. • The training on advanced laboratory analysis of mosquito samples to be conducted in 2021 in INRB by the London School of Hygiene and Tropical Medicine has been postponed to April/May 2022.
Meetings	• VectorLink DRC financially and technically supported NMCP to plan and hold a national meeting of the Vector Control Working Group (*Groupe de Travail Lutte Anti-Vectorielle,* or GTLAV) at Carita Congo Hotel on October 8, 2021. This is a multi-sectoral group composed of participants from NMCP; the ministries of Health, Agriculture, Environment and Hygiene, and Plant Protection; the directorates of Disease and Pharmacy; and universities, research institutions, civil society organizations, mining companies, and technical partners. Due to COVID-19, the coordinating team of GTLAV organized one of the two bi-annual meetings scheduled for 2021.
Molecular analysis and office development	• In 2021, the project bought a backup generator at the INRB entomological unit for the molecular laboratory dedicated to entomology in 2020. This reinforcement enabled the project to complete molecular data on-schedule without the need for an addendum report. • The project supported INRB to set up two offices and one stockroom in another converted shipping container.

4. DISCUSSION

The climate of Lodja province in central DRC is particularly favorable for the proliferation of malaria vectors, with year-round high temperatures and only a short dry season. *An. gambiae* s.l. biting rates were high in this province throughout the year. The annual EIRs of 242.56 infectious bites per person in Lodja and 358.34 infectious bites per person per year in Kimpese highlight the extremely high year-round malaria transmission risk in these provinces. However, the EIR was zero in Inongo due to low biting rates and no sporozoite infection of *An. gambiae* s.l. collected. In high transmission areas such as Lodja and Kimpese, multiple interventions (e.g., indoor residual spraying) in addition to ITNs can help reduce malaria transmission.

Insecticide susceptibility tests showed that pyrethroid resistance is widespread. In all sites, except Lodja, *An. gambiae* s.l. were resistant to permethrin, deltamethrin, and alpha-cypermethrin. Resistance intensity varied by site and by insecticide but was usually moderate or high. For example, resistance to deltamethrin was recorded in 10 sites, possible resistance observed in one site (Lodja), and susceptibility observed in one site (Katana). The intensity of deltamethrin resistance was moderate in eight sites (Inongo, Kapolowe, Kingasani, Karawa, Pawa, Mbandaka, Mweneditu, and Lisala) and high in one site (Kimpese).

Despite uncertainty regarding the impact of pyrethroid resistance, WHO states that, "When resistance is confirmed at the 5× and especially at the 10× concentrations, operational failure is likely." Throughout the DRC monitoring sites, resistance to the three most common pyrethroids used on ITNs was observed at the 5× and 10× concentrations, therefore making it highly likely that pyrethroid ITNs are no longer providing optimal protection against malaria (though this needs to be confirmed through epidemiological data).

The high intensity of pyrethroid resistance indicates that NMCP should consider new types of ITNs for future distribution campaigns. In all sites, bioassays with permethrin following pre-exposure to PBO 4% in WHO tube tests showed an increase in mortality compared with permethrin alone. Despite this, mortality was still <90% in nine sites, there was an increase in mortality with deltamethrin and alpha-cypermethrin following pre-exposure to PBO 4% in all 12 sites. Increases in deltamethrin mortality were particularly significant in Inongo, Kingasani, Mbandaka, Mweneditu, Lisala, and Boende. Despite an increase in mortality after pre-exposure to PBO, mortality was still <90% in Kimpese, Kapolowe, Karawa, and Pawa for deltamethrin and in Kimpese, Kapolowe, Kingasani, Karawa, Pawa, Mweneditu, and Lisala for alpha-cypermethrin.

The general increase in mortality when a PBO synergist was used indicates that ITNs containing PBO may provide greater control, although full susceptibility was not restored. A better option may be Interceptor G2 ITNs, as susceptibility to chlorfenapyr was recorded in seven sites (Kimpese, Inongo, Kingasani, Mbandaka, Mweneditu, Lisala, and Boende) and resistance in two sites (Karawa and Pawa) where testing was conducted. The results of PBO and Interceptor G2 ITN durability studies being conducted by VectorLink over three years in Sud Ubangi and Tanganyika (PBO) and Nord Ubangi (Interceptor G2) provinces will provide additional data to inform the NMCP and stakeholders.

To continue decentralized insecticide resistance testing, refresher training should be organized every two or three years in Kinshasa to bring all field teams together for standardized testing. Training would also be needed if additional bioassay methods were to be implemented, such as chlorfenapyr susceptibility testing using CDC bottle bioassays, at test that was not conducted in sites with no INRB supervision.

5. ANNEX

Table 12: Monthly HBR of Malaria Vectors Collected Indoors and Outdoors by HLC in Lodja

Lodja, January–December 2021															
Species	**Location**	**Variables**	**Jan**	**Feb**	**Mar**	**Apr**	**May**	**Jun**	**July**	**Aug**	**Sep**	**Oct**	**Nov**	**Dec**	**2021 Total**
An. gambiae s.l.	Indoor	Total collected	206	226	244	222	327	70	63	98	62	53	50	84	1,705
		nbr person-nights	8	8	8	8	8	8	8	8	8	8	8	8	96
		HBR/night	**26**	**28**	**31**	**28**	**41**	**9**	**8**	**12**	**8**	**7**	**6**	**11**	**18**
	Outdoor	Total collected	366	511	400	381	657	122	122	481	162	154	105	250	3,711
		nbr person-nights	8	8	8	8	8	8	8	8	8	8	8	8	96
		HBR/night	**46**	**64**	**50**	**48**	**82**	**15**	**15**	**60**	**20**	**19**	**13**	**31**	**39**
An. funestus s.l.	Indoor	Total collected	2	0	3	0	5	0	4	0	1	3	1	0	19
		nbr person-nights	8	8	8	8	8	8	8	8	8	8	8	8	96
		HBR/night	**0**	**0**	**0**	**0**	**1**	**0**	**1**	**0**	**0**	**0**	**0**	**0**	**0**
	Outdoor	Total collected	1	8	5	1	6	1	6	0	1	5	3	1	38
		nbr person-nights	8	8	8	8	8	8	8	8	8	8	8	8	96
		HBR/night	**0**	**1**	**1**	**0**	**1**	**0**	**1**	**0**	**0**	**1**	**0**	**0**	**0**
An. paludis	Indoor	Total collected	19	17	29	29	29	30	29	9	35	31	35	36	328
		nbr person-nights	8	8	8	8	8	8	8	8	8	8	8	8	96
		HBR/night	**2**	**2**	**4**	**4**	**4**	**4**	**4**	**1**	**4**	**4**	**4**	**5**	**3**
	Outdoor	Total collected	73	73	93	95	77	147	131	42	164	88	128	156	**1,267**
		nbr person-nights	8	8	8	8	8	8	8	8	8	8	8	8	96
		HBR/night	**9**	**9**	**12**	**12**	**10**	**18**	**16**	**5**	**21**	**11**	**16**	**20**	**13**

Note: HBR=human biting rate, *nbr=knockdown resistance*

Table 11: Monthly HBR of Malaria Vectors Collected Indoors and Outdoors by HLC in Kimpese

Kimpese, January–December 2021															
Species	**Location**	**Variables**	**Jan**	**Feb**	**Mar**	**Apr**	**May**	**Jun**	**July**	**Aug**	**Sep**	**Oct**	**Nov**	**Dec**	**2021 Total**
An. gambiae s.l.	Indoor	Total collected	35	40	64	24	42	11	8	5	1	8	36	101	375
		nbr person-nights	8	8	8	8	8	8	8	8	8	8	8	8	96
		HBR/night	**4**	**5**	**8**	**3**	**5**	**1**	**1**	**1**	**0**	**1**	**5**	**13**	**4**
	Outdoor	Total collected	37	50	72	50	32	7	3	2	4	10	54	143	464
		nbr person-nights	8	8	8	8	8	8	8	8	8	8	8	8	96
		HBR/night	**5**	**6**	**9**	**6**	**4**	**1**	**0**	**0**	**1**	**1**	**7**	**18**	**5**
An. funestus s.l.	Indoor	Total collected	183	81	100	137	145	43	46	27	27	29	46	111	975
		nbr person-nights	8	8	8	8	8	8	8	8	8	8	8	8	96
		HBR/night	**23**	**10**	**13**	**17**	**18**	**5**	**6**	**3**	**3**	**4**	**6**	**14**	**10**
	Outdoor	Total collected	152	145	126	119	135	44	26	34	34	27	89	168	1,099
		nbr person-nights	8	8	8	8	8	8	8	8	8	8	8	8	96
		HBR/night	**19**	**18**	**16**	**15**	**17**	**6**	**3**	**4**	**4**	**3**	**11**	**21**	**12**
An. paludis	Indoor	Total collected	0	0	0	0	0	0	0	0	0	0	0	0	0
		nbr person-nights	8	8	8	8	8	8	8	8	8	8	8	8	96
		HBR/night	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
	Outdoor	Total collected	0	0	0	0	0	0	0	1	0	1	0	0	2
		nbr person-nights	8	8	8	8	8	8	8	8	8	8	8	8	96
		HBR/night	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**

Note: HBR=human biting rate, *nbr=knockdown resistance*

Table 10: Monthly HBR of Malaria Vectors Collected Indoors and Outdoors by HLC in Inongo

Inongo, January–December 2021															
Species	**Location**	**Variables**	**Jan**	**Feb**	**Mar**	**Apr**	**May**	**Jun**	**July**	**Aug**	**Sep**	**Oct**	**Nov**	**Dec**	**2021 Total**
An. gambiae s.l.	Indoor	Total collected	13	4	1	2	1	0	0	2	7	1	0	1	32
		nbr person-nights	8	8	8	8	8	8	8	8	8	8	8	8	96
		HBR/night	**2**	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**1**	**0**	**0**	**0**	**0**
	Outdoor	Total collected	19	2	1	1	1	0	0	3	3	1	0	2	33
		nbr person-nights	8	8	8	8	8	8	8	8	8	8	8	8	96
		HBR/night	**0**	**1**	**3**	**2**	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**1**
An. funestus s.l.	Indoor	Total collected	0	0	0	0	0	0	0	0	0	0	0	0	0
		nbr person-nights	8	8	8	8	8	8	8	8	8	8	8	8	96
		HBR/night	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
	Outdoor	Total collected	1	0	0	0	0	0	0	0	0	0	0	1	2
		nbr person-nights	8	8	8	8	8	8	8	8	8	8	8	8	96
		HBR/night	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
An. paludis	Indoor	Total collected	103	77	100	106	117	128	102	103	103	135	118	104	1,296
		nbr person-nights	8	8	8	8	8	8	8	8	8	8	8	8	96
		HBR/night	**13**	**10**	**13**	**13**	**15**	**16**	**13**	**13**	**13**	**17**	**15**	**13**	**14**
	Outdoor	Total collected	69	76	103	110	98	124	101	100	89	99	89	118	1,176
		nbr person-nights	8	8	8	8	8	8	8	8	8	8	8	8	96
		HBR/night	**9**	**10**	**13**	**14**	**12**	**16**	**13**	**13**	**11**	**12**	**11**	**15**	**12**

Note: HBR=human biting rate, *nbr=knockdown resistance*