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THE PMI VECTORLINK PROJECT CAMEROON

ANNUAL ENTOMOLOGY REPORT

OCTOBER 2019 – SEPTEMBER 2020

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ACRONYMS

AI	active ingredient
BTC	Biotechnology Center
CDC	(U.S.) Centers for Disease Control and Prevention
CRID	Center for Research in Infectious Diseases
EIR	Entomological Inoculation Rate
ELISA	Enzyme-Linked Immunosorbent Assay
HBI	Human Blood Index
HBR	Human Biting Rate
HLC	Human Landing Catch
IRD	Indoor Resting Density
ITN	insecticide-treated net
kdr	Knock Down Resistance
LT	light trap
NMCP	National Malaria Control Program
OCEAC	Organization for the Coordination of Endemic Diseases Control in Central Africa
PBO	Piperonyl butoxide
PCR	Polymerase Chain Reaction
PMI	(U.S.) President's Malaria Initiative
PSC	Pyrethrum Spray Catch
SOP	standard operating procedure
USAID	United States Agency for International Development
WHO	World Health Organization

EXECUTIVE SUMMARY

From October 2019 to September 2020, the U.S. President's Malaria Initiative (PMI) VectorLink Project conducted malaria vector surveillance in five sentinel sites in Cameroon. In all sites, longitudinal vector surveillance was conducted every two months from October 2019 to March 2020 and monthly from June to September 2020, for a total of seven collection efforts at each site. Field collections were suspended in April and May 2020 per PMI guidance following the COVID-19 pandemic. Three collection methods—human landing catches (HLCs), pyrethrum spray catches (PSCs), and U.S. Centers for Disease Control and Prevention Light Traps (CDC LTs)—were used to collect adult mosquitoes in households and assess vector composition, human biting rate (HBR), endophagic index, indoor resting density, parity rate, human blood index (HBI), infection rate, and entomological inoculation rate (EIR). In addition, insecticide susceptibility, intensity of resistance, and synergist assays with piperonyl butoxide (PBO) were conducted.

High diversity of the *Anopheles* species was collected during monthly longitudinal surveillance. Among these, 10 were involved in malaria transmission including *An. gambiae* s.l., *An. funestus* s.l., *An. nili*, *An. moucheti*, *An. demeillonni*, *An. pharoensis*, *An. ziemanni*, *An. multicinctus, An. rufipes,* and *An. marshallii*. Identification of *An. gambiae* complex and *An. funestus* group using Polymerase Chain Reaction (PCR) revealed the presence of three species of the *An. gambiae* complex: *An. gambiae* s.s. (26.5%), *An. coluzzii* (68.8%) and *An. arabiensis* (4.6%). One hybrid of *An. gambiae*/*An. coluzzii* was also found (0.04%) in Mangoum. Two species of the *An. funestus* group were identified in Gounougou and Simatou: *An. funestus* s.s. (95.5%) and *An. leesoni* (4.5%); only *An. leesoni* was recorded in Nyabessang.

The mean HBR of the *Anopheles* mosquitoes varied from 10.2 bites/person/night (b/p/n) (Mangoum) to 116.3 b/p/n (Simatou). The highest HBR was observed in Simatou, where rice cultivation zones host permanent suitable vector breeding habitats. Early morning continuous biting was observed for *An. gambiae* s.l., particularly in the Northern sites of Gounougou and Simatou where vector biting occurred until 8 a.m. The average *Anopheles* indoor resting density across sites was 35.1 females/room/night. The overall parity rate ranged from 65% (Nyabessang) to 77% (Simatou) and the HBI ranged from 47.0% (Simatou) to 94.0% (Mangoum), suggesting that the vectors were old enough to transmit *Plasmodium* parasites and also bite more humans than animals. The endophagic indexes of *An. gambiae* s.l. were 0.5 in Gounougou and 0.52 in Simatou, indicating that *An. gambiae* s.l. bites equally indoors and outdoors in these two sites. The endophagic indexes were 0.46 in Mangoum, 0.43 in Nyabessang, and 0.35 in Bonabéri, showing that *An. gambiae* s.l. bites more outdoors in these three sites. The monthly EIR was 17.4 infected bites/person/month (ib/p/m) in Gounougou, 22.8 ib/p/m in Simatou, 8.70 ib/p/m in Mangoum, 12.8 ib/p/m in Nyabessang, and 6.2 ib/p/m in Bonabéri. These results raise concerns as the parasite was detected in various *Anopheles* species collected.

Resistance of *An. gambiae* s.l. to the three pyrethroids tested (permethrin, deltamethrin, alpha-cypermethrin) was recorded in all sites. The intensity of this resistance varied by site and insecticide. High resistance to deltamethrin, permethrin, and alpha-cypermethrin was observed in Gounougou, Simatou, Nyabessang, and Mangoum; in Bonabéri, moderate resistance to permethrin and low resistance to alpha-cypermethrin was observed. Furthermore, pre-exposure to PBO before testing with pyrethroids did partially increase mortality of *An. gambiae* s.l., except in Bonabéri with alpha-cypermethrin where susceptibility (100% mortality) was recorded.

An. gambiae s.l. was resistant to pirimiphos-methyl only in Mangoum. Susceptibility to bendiocarb was recorded in Simatou, Gounougou and Bonabéri and resistance in Mangoum and Nyabessang. Susceptibility to clothianidin and chlorfenapyr was recorded in all five sites, allowing several options for vector control tool selection. Furthermore, target site knockdown resistance (*Kdr)* west and east (*kdr-e* and *kdr-w*), *Ace-1,* and N1575Y were found to be involved in the insecticide resistance of the vectors of the different sites.

These results from entomological monitoring will support the NMCP with vector control and strategy decision making, particularly with the review of its insecticide resistance management plan and with the targeted distribution of PBO insecticide-treated nets (ITNs) and dual active ingredient (AI) ITNs in the country.

1. INTRODUCTION

Malaria remains a public health problem in Cameroon and is one of the main causes of morbidity and mortality with nearly three million cases and 4,500 deaths recorded in health facilities in 2019 (National Malaria Control Program, 2019). Children under 5 years of age account for around 60% of malaria cases and deaths while morbidity among pregnant women increased from 12.7% in 2013 to 19.6% in 2019 (NSP 2019-2023, NMCP, 2019). Given the scale of the problem, the Ministry of Public Health and its partners are implementing high-impact interventions to reduce malaria morbidity and mortality. These include i) the free distribution of insecticide-treated nets (ITNs) during campaigns and antenatal consultations to pregnant women, ii) intermittent preventive treatment to pregnant women during antenatal consultations, iii) seasonal chemoprophylaxis of malaria in children aged 3 to 59 months, and iv) free treatment of uncomplicated and severe malaria in children under 5 years old.

In September 2017, Abt Associates was awarded the five-year U.S. President's Malaria Initiative (PMI) VectorLink Project to conduct entomological surveillance in Cameroon. Since October 2018, PMI VectorLink Cameroon has carried out entomological monitoring in five sentinel sites located in various regions representing different ecologies in the country. VectorLink works in close collaboration with the National Malaria Control Program (NMCP) and three research institutions—the Biotechnology Center (BTC), the Center for Research in Infectious Diseases (CRID), and the Organization for the Coordination of Endemic Diseases Control in Central Africa (OCEAC)—to conduct longitudinal surveillance and insecticide resistance monitoring.

The data presented in this report aim to support and guide the NMCP and other stakeholders on the selection of appropriate malaria vector control tools and strategies.

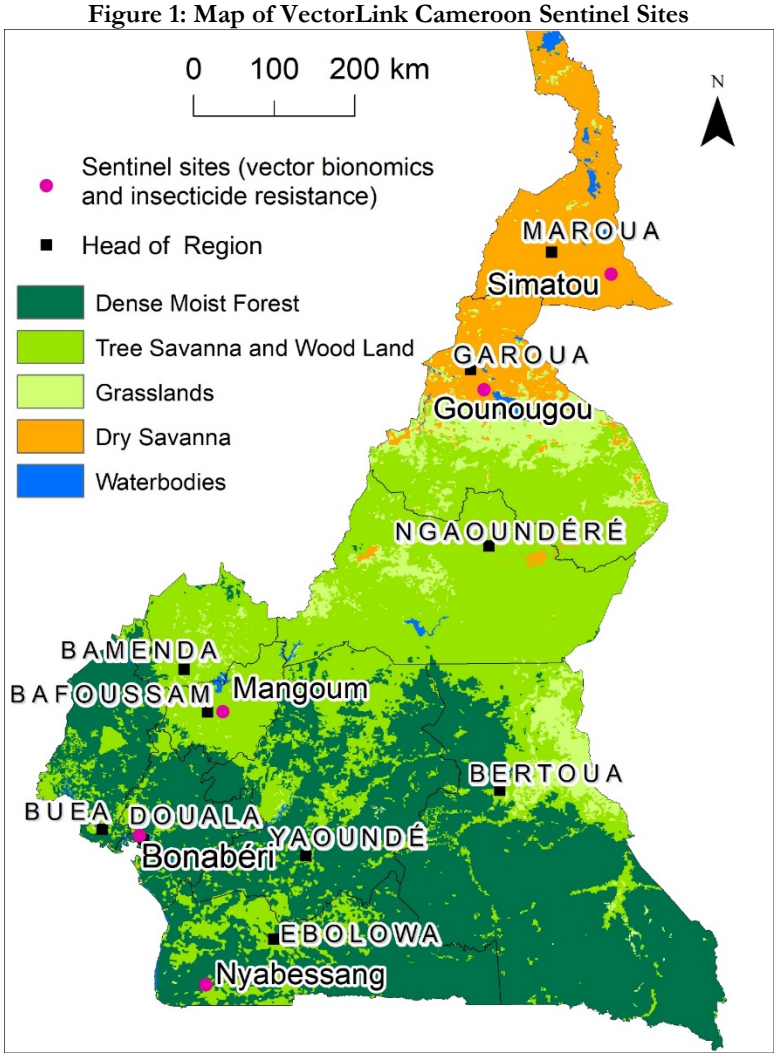
2. METHODS

2.1 STUDY SITES

From October 2019 to September 2020, VectorLink Cameroon conducted entomological surveillance in five sentinel sites—Gounougou, Simatou, Nyabessang, Mangoum, and Bonabéri (Figure 1). Gounougou and Simatou are in the dry savannah and Sahelian zones of the North and Far North regions, Mangoum is in the wet, savannah zone of the West region, Nyabessang is in the forest area of the South region, and Bonabéri is in the coastal zone of the Littoral region.

In all sites, adult mosquito collections were conducted every two months from October 2019 to March 2020 and monthly from June to September 2020, for a total of seven collection efforts during the reporting period. Field collections were suspended in April and May 2020 due to the COVID-19 pandemic and in accordance with PMI guidance. Insecticide resistance monitoring was also conducted at each site in September during the rainy season.

Figure 1: Map of VectorLink Cameroon Sentinel Sites



2.2 LONGITUDINAL MONITORING OF MALARIA VECTORS

VectorLink Cameroon collected adult mosquitoes using human landing catches (HLCs), pyrethrum spray catches (PSCs), and U.S. Centers for Disease Control and Prevention Light Traps (CDC LTs) in all sentinel sites following the PMI Standard Operating Procedures (SOPs)[1]. For each collection method, the same houses were used each month for collections and seven collections were completed in each site during the reporting period.

Table 1 provides additional information on mosquito collection methods used and Table 2 summarizes the indicators calculated based on the number of mosquitoes captured through each collection method.

Table 1: Adult Mosquito Collection Methods for Vector Surveillance

Collection Method	Time	Collection Location	Frequency	Sample
HLCs	6:00 p.m.–8:00 a.m.	Indoors and outdoors	Two nights per site	Three houses per site (same houses every month)
PSCs	6:00 a.m.–8:00 a.m.	Indoors	Two days per site	Twenty houses per site (the same houses most of the time)
CDC LTs	6:00 p.m.–6:00 a.m.	Indoors (baited) and outdoors (no bait)	Two nights per site	Four houses per site (same houses every month)

Table 2: Vector Surveillance Indicators by Collection Method

Collection Method	Indicator	Definition
HLC	Human Biting Rate	Mean number of bites per person per night
	Peak biting time	Hour of highest human biting rate
	Parity Rate	Percentage of parous mosquitoes/total dissected
	Exophagic Rate	Proportion of mosquitoes biting outside
	Endophagic Rate	Proportion of mosquitoes biting inside
PSC	Indoor Resting Density	Mean number of mosquitoes per room per day
	% of fed females	Number of fed mosquitoes / total collected by PSC
	Human Blood Index	Number of female mosquitoes that have taken human bloodmeal / total female mosquitoes with bloodmeal
CDC LT	Indoor/Outdoor Density	Mean number of mosquitoes collected indoors or outdoors per trap per night

2.2.1 HUMAN LANDING CATCHES

HLCs were performed indoors and outdoors in three houses for two consecutive nights, to collect adult mosquitoes landing on human baits from 6:00 p.m. to 8:00 a.m. With legs exposed to attract host-seeking mosquitoes, two human baits serving as mosquito collectors were seated about 1.5-2 meters from each other indoors while another two were seated outdoors. The two teams of 12 collectors each worked in two shifts—6:00 p.m. to 12:00 a.m. and 12:00 a.m. to 8:00 a.m. The collectors swapped positions (indoor and outdoor) every hour. The doors of the houses were kept closed when collections were underway. The collectors used

[1]Complete SOPs can be found here: https://pmivectorlink.org/resources/tools-and-innovations/

flashlights and hemolysis tubes to collect mosquitoes that landed on their legs before the mosquitoes could bite. The tubes were covered with cotton after individual collection of mosquitoes. The teams transferred the mosquitoes hourly to custom-made bags for a total of 14 hours. Mosquitoes collected all night and at hourly intervals were identified morphologically.

2.2.2 Pyrethrum Spray Catches

PSCs were carried out during morning hours, between 6:00 a.m. and 8:00 a.m. for two consecutive days in 20 sleeping rooms. White cloth sheets were placed on the floor from wall to wall in sampled rooms. After closing the windows and doors and covering or removing drinking water and food items, the rooms were sprayed with a commercial pyrethroid + piperonyl butoxide (PBO) insecticide. For houses with open eaves, collectors sprayed from outside through the eaves before entering and spraying indoors. Ten minutes after spraying, all mosquitoes knocked down by the chemical were collected using the white sheets. The mosquitoes were kept in Petri dishes and then sorted by species using an identification key. The abdominal status of all female anophelines was determined, and individuals were sorted into four categories: unfed, blood-fed, half-gravid, and gravid. To determine blood meal status, female *Anopheles* mosquitoes were classified according to their abdomen status and were kept individually in labeled Eppendorf tubes containing silica gel.

2.2.3 CDC Light Traps

CDC light traps were installed for two consecutive nights, one indoors and one outdoors, of four houses (8 traps per night) in each site at each collection period between 6:00 p.m. and 6:00 a.m. The traps were suspended 1.5 meters above the ground. Indoors, the trap was installed in a bedroom used for sleeping by at least one household member and containing at least one treated mosquito net (typically received from the different ITN distribution campaigns) and in use, and near the feet of the sleeper. Outdoors, the trap is set un-baited near the house of collection. Two volunteers were recruited to check on the traps during collection nights. The next morning, *Anopheles* collected were identified and the ovaries of subsamples of unfed *Anopheles* that were still alive were dissected.

2.2.4 Identification of Malaria Vectors

All mosquitoes were identified morphologically using identification keys (Coetzee, 2020). All *Anopheles* specimens collected were labelled and stored individually over silica gel in Eppendorf tubes for further processing. All samples collected were sent to CRID for molecular analysis.

2.2.5 PCR Identification of Members of *An. gambiae* Complex and *An. funestus* Group

Polymerase chain reaction (PCR) assays were carried out on mosquito samples collected to identify members of the *An. gambiae* complex and *An. funestus* group at CRID, Yaoundé. PCR was conducted on approximately 100 *An. gambiae* s.l. and 25 *An. funestus* s.l. per month using the *An. gambiae* species-specific single interspersed element (SINE) PCR (Santolamazza *et al.* 2008). In coastal sites where other species such as *An. melas* are present the team used the PCR-RFLP protocol described by Fanello et al, 2002. *An. funestus* group species was determined using a cocktail PCR with addition of the *An. rivulorum-like* primers. gDNA from randomly selected mosquitoes were processed per each location period as described by Koekemoer *et al, 2002*. All PCR products were run via electrophoresis through a 1.5% agarose gel with Midori Green® (Gene flow, UK) and visualized using ultraviolet light. The multiplex PCR assays (Koekemoer, *et al*., 2002) was used to determine members of the *An. funestus* group.

2.2.6 Blood Meal Assays

The source of the blood contained in the abdomen of resting mosquitoes collected by indoor PSCs were determined using a direct ELISA technique described by Beier *et al*, 1988. This technique simultaneously allows the identification of human, cow, pig chicken, goat, pig horse and dog blood. Peroxydase conjugated anti-bodies, as well as animal heterologous serum were obtained from Sigma (www.sigmaaldrich.com). After manipulation, absorbance at 414 nm were determined with an ELISA plate reader. Samples were considered as

positive if absorbance values exceed the mean plus three times the standard deviation of four negative control represented by unfed mosquitoes.

2.2.7 Determination of Parity Rate

To determine parity rate, the team dissected ovaries about 20% of the total of randomly selected hourly, unfed, female *Anopheles* collected using HLC and CDC-LT methods. Ovary dissection was done each month for seven months and the ovary status of the dissected mosquitoes was determined following the methods described by Detinova 1962, Detinova and Gillies 1964.All *Anopheles* and the carcasses of the dissected *Anopheles* were individually stored in labeled Eppendorf tubes containing silica gel. Mean parity rate was determined by dividing the number of parous females by the total number dissected and confirmed by observing the degree of coiling by the ovarian tracheoles (WHO, 2013).

2.2.8 *Plasmodium* Sporozoite Detection

To estimate the *Plasmodium* infection rate in the mosquito population, CRID performed enzyme-linked immunosorbent assays (ELISAs) for sporozoite antigen on a proportion of randomly selected mosquitoes collected from the field using all methods (HLC, PSC and CDC LT). An ELISA-CSP method described by Burkot et al and modified by Wirtz et al was used for sporozoite detection in the head and thorax of mosquitoes. This method uses a monoclonal antibody that recognizes a repetitive epitope on the circumsporozoite protein of *P. falciparu*m. *Plasmodium falciparum* sporozoite ELISA Reagent Kit (MRA-890) were obtained from BIE Resources (NIAID, NIH, USA). Lyophilized *P. falciparum* monoclonal antibody was reconstituted prior utilization using glycerol-water solution to achieve a final concentration of 0.5mg/ml. Similarly, all reagents including phenol red, 1X Phosphate Buffered Saline (PBS), Blocking Buffer (BB), Grinding Buffer, 1X PBS-Tween wash solution will be prepared before to start the manipulation following product information sheet provided with the MR4-890 kit. Diluted *P. falciparum* sporozoite proteins supplied by the Center for Disease Control (CDC, Atlanta, USA) were used as positive controls, while ground male mosquitoes were used as negative controls. Determination of positive samples will be done after reading optical densities (OD) at 405 nm on an ELISA plate reader (Biotek ELx800, Swindon, UK). Positive samples were determined by OD readings 2-fold greater than the negative controls and will be tested a second time for validation as we do regularly (1).

2.3 Insecticide Resistance Monitoring

2.3.1 Susceptibility Tests of *An. gambiae* s.l.

In September 2020, the team completed insecticide resistance monitoring in the five sentinel sites (Gounougou, Simatou, Mangoum, Nyabessang, and Bonabéri). *Anopheles gambiae* s.l. larvae and pupae were collected per site from different larval habitats, pooled, and reared to adulthood in the field laboratory. Insecticide susceptibility tests were conducted on two- to five-day old adult females using World Health Organization (WHO) tube tests. CDC bottle assays were used to test the susceptibility to chlorfenapyr. For each WHO susceptibility test and CDC bottle assay, two control groups of 20-25 female *An. gambiae* s.l. were used and tested similarly using paper impregnated with either silicone oil for pyrethroid or olive oil for organophosphate/carbamate controls for the WHO tube test. Bottles coated with acetone alone were used for the CDC bottle assays.

The diagnostic concentrations of permethrin (0.75%), deltamethrin (0.05%), alpha-cypermethrin (0.05%), bendiocarb (0.1%), and pirimiphos-methyl (0.25%) were tested in all sites. Resistance was defined following the WHO criteria, with less than 90% mortality indicating confirmed resistance, between 90-97% mortality indicating possible resistance, and greater than 98% indicating susceptibility. When insecticide resistance was confirmed, resistance intensity (high, moderate, and low) was also tested at five- and ten-times the diagnostic concentration of permethrin, deltamethrin, and alpha-cypermethrin.

Synergist assays with piperonyl butoxide (PBO) were conducted for deltamethrin, permethrin, and alpha-cypermethrin according to the WHO tube test protocol to determine the involvement of P450s in pyrethroid

resistance. A high percentage mortality and/or reversal of susceptibility using PBO indicated probable involvement of enzyme activities such as P450s in this insecticide resistance mechanism.

Clothianidin-impregnated papers were treated locally at the dose of 2% using a protocol designed by VectorLink and the susceptibility testing was done as described above with a seven-day delay mortality recording. CDC bottles were treated with chlorfenapyr at a selected dose of 100 μg/bottle. The mosquitoes were exposed for one hour and the mortality was recorded up to three days. All tests, paper impregnation, and coating of bottles were conducted following PMI VectorLink SOPs.

2.3.2 Detection of Resistance Mechanisms

A subsample of 100 (dead and alive) randomly selected mosquitoes per sites, after exposure to insecticides were species identified and resistance mechanisms determined using PCR methods.

2.3.2.1 Target Site Resistance Mechanisms

Anopheles gambiae s.l. mosquitoes were examined for the presence of sodium channel mutations *kdr alleles* "west" and "east" (L1014F and L1014S) using relevant PCR protocols described by Martinez-Torres *et al*, 1998 and Ranson et al, 2000 already optimized at the CRID. The different amplicons were run on a 2% agarose gel and visualized under UV light, allowing the definition of the genetic profile of each mosquito sample (*kdr-w*, *kdr-e* or no *kdr*) from the size of the amplicons observed. The presence of the additional *kdr* allele N1575I shown to increase resistance in the presence of L1014F in *An. gambiae* s.l. were monitored using a TaqMan assay (Jones *et al*, 2012). To assess the direct involvement of *kdr* in pyrethroid/DDT resistance, the team genotyped a set of mosquitoes (50 dead and 50 alive) mosquitoes tested for susceptibility.

The ace-1 gene mutation was detected by PCR using the protocol of Weill et al, 2004. Extracted DNA was amplified by PCR with Ex3AGdir and Ex3AGrev oligonucleotide primers. The PCR amplification products were analyzed by electrophoresis onto a 2% agarose gel and visualized under UV light. The two primers produced a 403 bp fragment, which is undigested by AluI for susceptible homozygous mosquitoes (SS) and cut into two fragments (253 bp and 150 bp) for homozygous resistant (RR). Heterozygous individuals (RS) display a combined pattern.

2.3.2.2 Metabolic Resistance Enzyme Detection

The gene expression patterns of key detoxification genes which have previously been detected as over-expressed in populations of malaria vectors in Cameroon were assessed in each site. Five cytochrome P450s and one GST genes were assessed using qRT-PCR. The selected genes are: *An. gambiae* and *An. coluzzii* (CYP6P3, CYP6M2, CYP9K1, CYP6P5, CYP6P4, GSTe2) (Fossog *et al*, 2013), *An. arabiensis* (CYP6P3, CYP6M2, CYP9K1, CYP6P5, CYP6P4, GSTe2) (Witzig *et al*, 2013), *An. funestus* s.l. (CYP6P9a, CYP6P9b, CYP6M7, CYP325A, CYP6P5, GSTe2) (Riveron *et al*, 2014). RNA was extracted from 3 biological replicates (pool of 15 specimen) of resistant *An. mosquitoes* (R) and that of control unexposed (C), and the fully susceptible laboratory strain of the respective species (S). The relative expression and fold change of each target gene in R and C relative to S was calculated according to the 2-ΔΔCT method, incorporating PCR efficiency after normalization with the housekeeping genes RSP7 (ribosomal protein S7, AGAP010592).

3. RESULTS

3.1 LONGITUDINAL MONITORING

From October 2019 to September 2020, mosquitoes were collected in five sentinel sites across the country to assess vector species composition, density, behavior, and transmission.

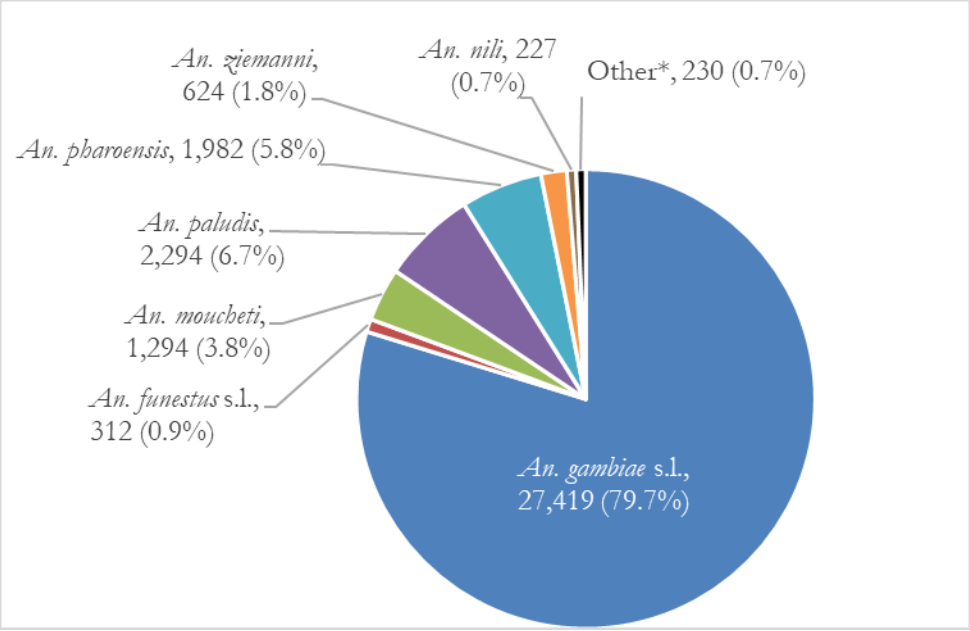
3.1.1 SPECIES COMPOSITION OF MOSQUITOES COLLECTED BY HLCS, PSCS, AND CDC LIGHT TRAPS ACROSS ALL SITES

From October 2019 to September 2020, a total of 34,382 *Anopheles* mosquitoes were collected by HLCs across the five sentinel sites. Twelve unique species were identified, with *An. gambiae* s.l. being the predominant species (79.7%), followed by *An. paludis* (6.7%), *An. pharoensis* (5.8%), and *An. moucheti* (3.8%) (Figure 2 and Table A1 in Annex A). *An. gambiae* s.l. were collected by HLCs at all five sentinel sites while *An. moucheti* and *An. nili* were only found at Nyabessang, which is surrounded by large rivers that offer suitable breeding sites for these two species.

A total of 17,763 mosquitoes, including 11,121 *Anopheles* mosquitoes belonging to 11 species, were caught using CDC LTs. *An. gambiae* s.l. (84.2%), *An. ziemanni* (8%), and *An. pharoensis* (3.2%) were the most abundant (Figure 3 and Table A2, Annex A).

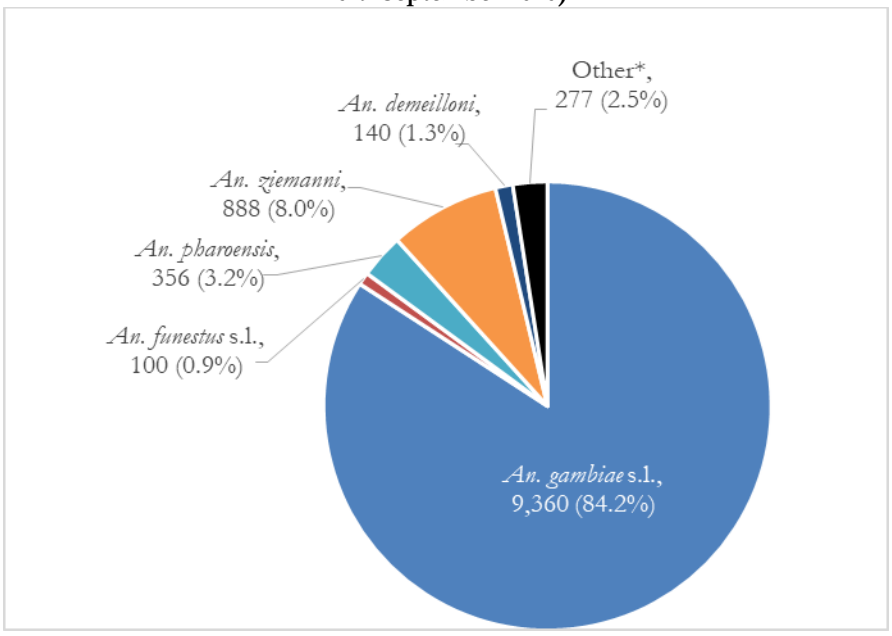
A total of 9,809 *Anopheles* mosquitoes belonging to nine different species were collected using PSCs from October 2019 to September 2020 in the five sites. *An. gambiae* s.l. (96.1%) was the main species collected and the only that was present at all five sites (Figure 4 and Table A3, Annex A).

Figure 2: Species Composition of *Anopheles* Mosquitoes Collected across All Sites Using HLCs (October 2019-September 2020)



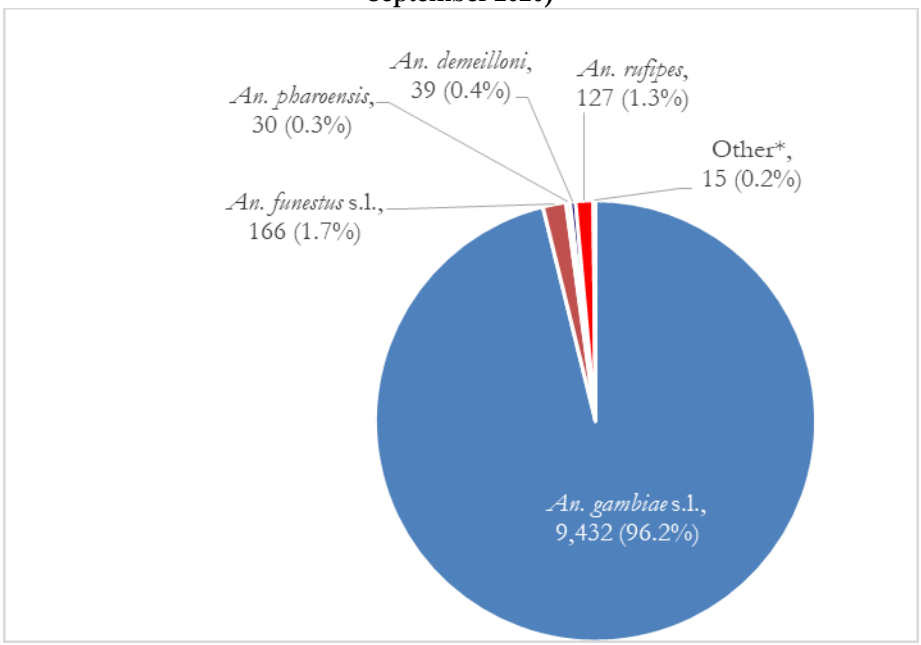
*Other: *An. marshallii* (119), *An. demeilloni* (64), *An. multicinctus* (38), *An. rufipes* (8), and *An. welcomei* (1).

Figure 3: Species Composition of *Anopheles* Mosquitoes Collected across All Sites Using CDC LTs (October 2019-September 2020)



*Other: *An. rufipes* (87), *An. moucheti* (65), *An. multicinctus* (10), *An. nili* (7), and *An. marshallii* (2).

Figure 4: Species Composition of *Anopheles* Mosquitoes Collected across All Sites Using PSCs (October 2019-September 2020)



*Others: *An. ziemanni* (6), *An. paludis* (5), *An. moucheti* (3), and *An. multicinctus* (1).

3.1.2 Species Composition of Mosquitoes Collected by HLCs, PSCs, and CDC Light Traps By Site

Simatou

In Simatou, 19,534 *Anopheles* mosquitos were collected using HLCs. *An. gambiae* s.l. (87.3%) was the most abundant (Figure 5). Among those collected by CDC LTs, 82.8% were *An. gambiae* s.l.; for PSCs, 96.6% were *An. gambiae* s.l. (Figures 6 and 7).

Figure 5: Species Composition of *Anopheles* Mosquitoes Collected in Simatou Using HLCs

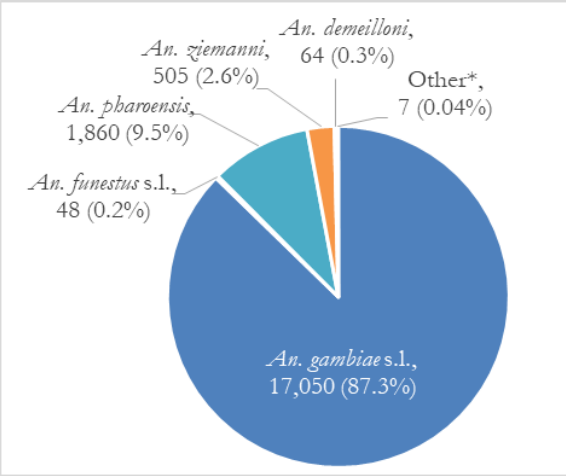


Figure 6: Species Composition of *Anopheles* Mosquitoes Collected in Simatou Using CDC LTs

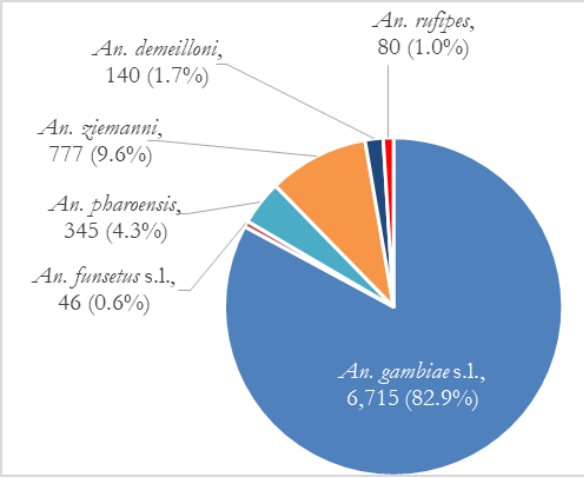
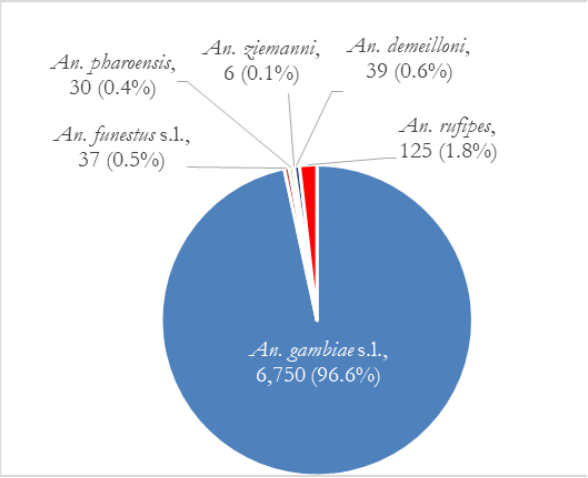


Figure 7: Species Composition of *Anopheles* Mosquitoes Collected in Simatou Using PSCs

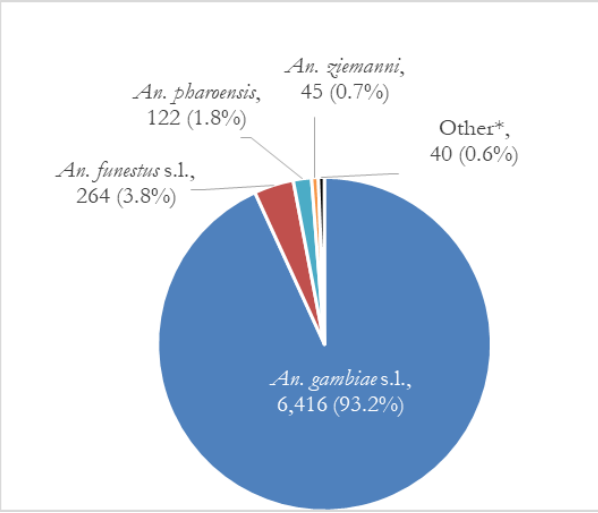


*Other: *An. rufipes* (6), and *An. welcomei* (1)

Gounougou

In Gounougou, *An. gambiae* s.l. represented 93.16% (6,416) of the 6,887 total *Anopheles* species collected using HLCs from October 2019 to September 2020. *An. funestus* (264, 3.83%) were also collected (Figure 8). A total of 2,367 and 2,615 *Anopheles* mosquitos were collected in Gounougou through CDC LTs and PSCs, respectively. For both methods, *An. gambiae* s.l. was the main vector collected, representing 91.9% (n=2,177) of the total vectors collected for CDC LTs and 94.2% (n=2,483) for PSCs (Figures 9 and 10).

Figure 8: Species Composition of *Anopheles* Mosquitoes Collected in Gounougou Using HLCs



*Other: *An. rufipes* (2) and *An. multicinctus* (38).

Figure 9: Species Composition of *Anopheles* Mosquitoes Collected in Gounougou Using CDC LTs

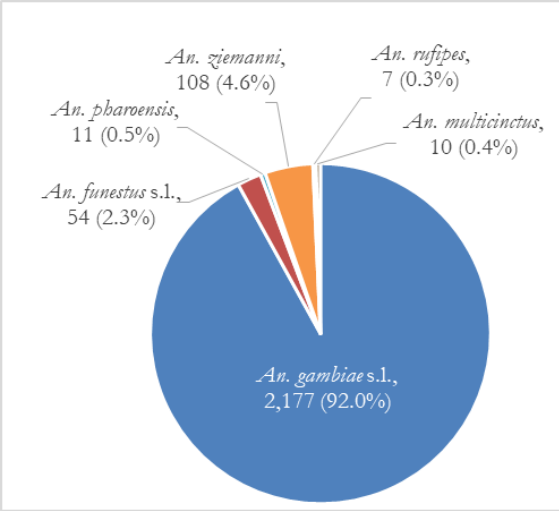
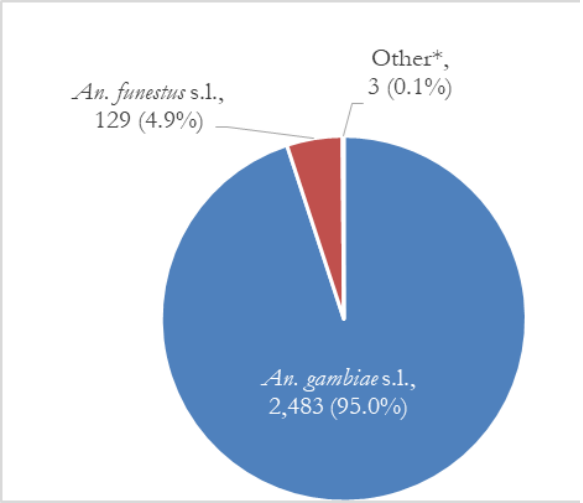


Figure 10: Species Composition of *Anopheles* Mosquitoes Collected in Gounougou Using PSCs



*Other: *An. rufipes* (2) and *An. multicinctus* (1).

Mangoum

In Mangoum, *An. gambiae* s.l. and *An. ziemanni* were the only *Anopheles* species collected using the three collection methods from October 2019 to September 2020. *An. gambiae* s.l. represented more than 95% of the total *Anopheles* mosquitoes collected across all three methods (Figures 11-13).

Figure 11: Species Composition of *Anopheles* Mosquitoes Collected in Mangoum Using HLCs

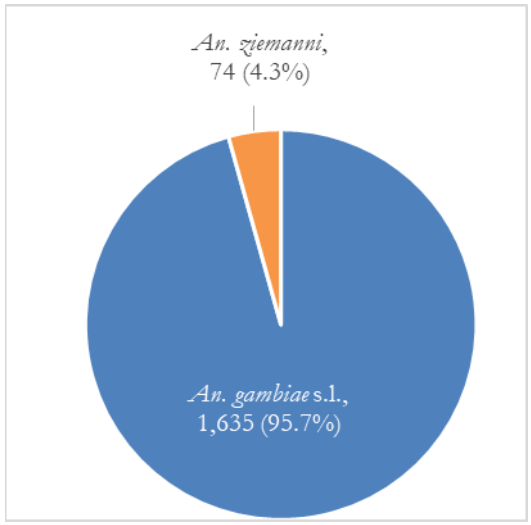


Figure 12: Species Composition of *Anopheles* Mosquitoes Collected in Mangoum Using CDC LTs

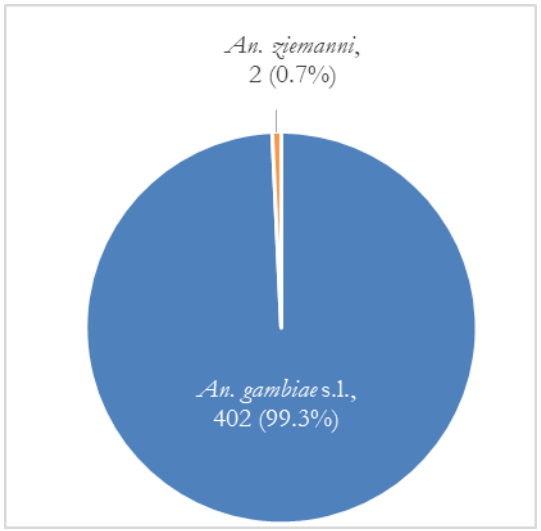
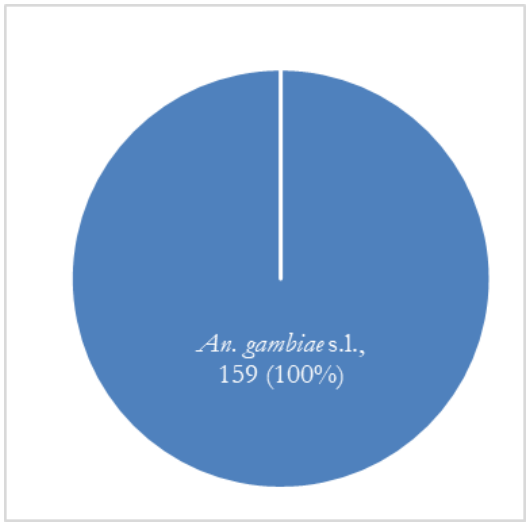


Figure 13: Species Composition of *Anopheles* Mosquitoes Collected in Mangoum Using PSCs



Nyabessang

Nyabessang is the only site where *An. moucheti* and *An. nili* were collected using HLCs, CDC LTs, and PSCs (Figure 14-15). *An. nili* was not collected using PSCs (Figure 16).

Figure 14: Species Composition of *Anopheles* Mosquitoes Collected in Nyabessang Using HLCs

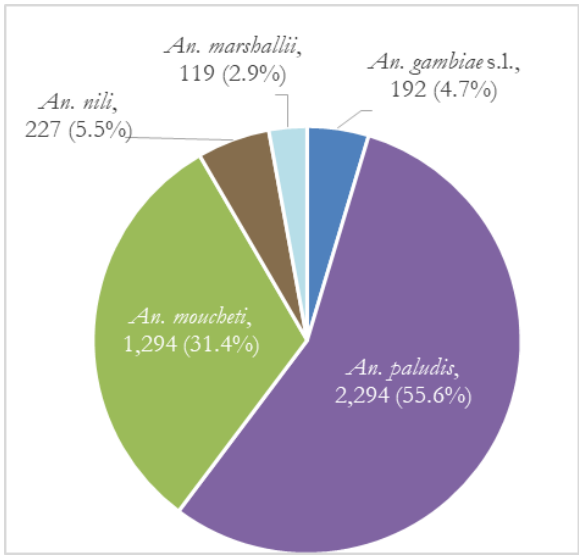


Figure 15: Species Composition of *Anopheles* Mosquitoes Collected in Nyabessang Using CDC LTs

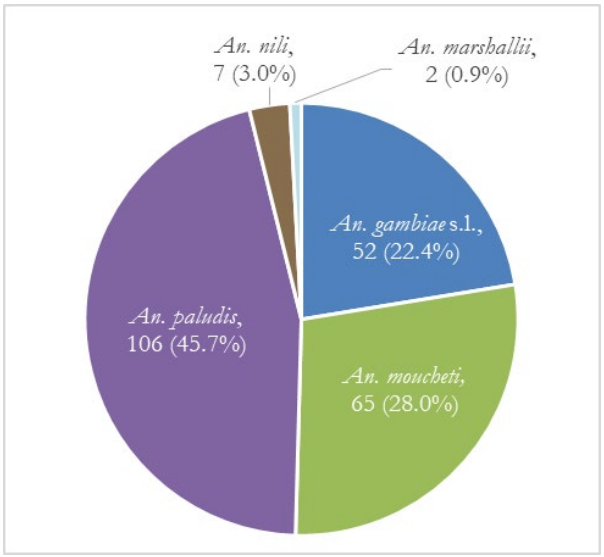
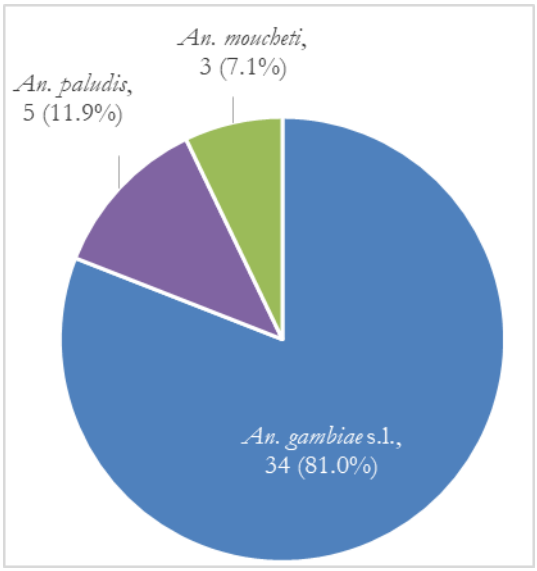


Figure 16: Species Composition of *Anopheles* Mosquitoes Collected in Nyabessang Using PSCs



Bonabéri

Bonabéri recorded the fewest number of *Anopheles* mosquitoes compared to the other four sites. *An. gambiae* s.l. represented the only *Anopheles* species collected using the three methods (100%): HLC (2,126), CDC LTs (14), and CDC LTs (6).

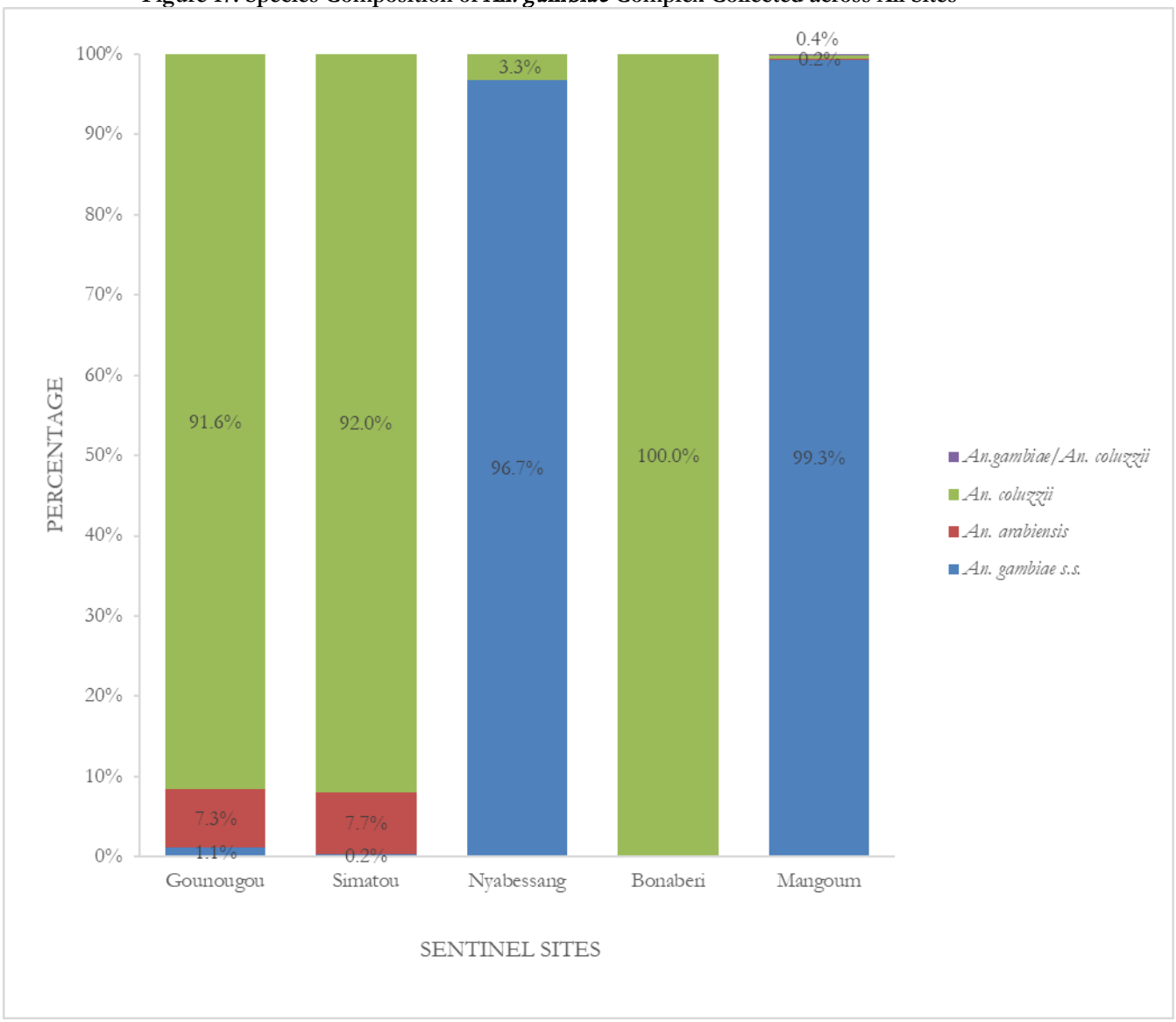
3.1.2 Species Composition of *An. gambiae* Complex and *An. funestus* Group

A total of 2,779 *An. gambiae* s.l. and 110 *An. funestus* s.l. were tested by PCR for molecular identification of the sub-species of each complex (Table A4, Annex A).

An. gambiae Complex

Of the total mosquitoes analyzed across the five sites, three species from the *An. gambiae* complex were identified: *An. gambiae* s.s. (26.5%), *An. coluzzii* (68.8%), and *An. arabiensis* (4.6%). One hybrid of *An. gambiae*/*An. coluzzii* (0.04%) was also found in Mangoum. In total, 866 *An. gambiae* s.l. from Simatou, 831 from Gounougou, 552 from Mangoum, 183 from Nyabessang, and 347 from Bonabéri underwent species identification by PCR. *An. gambiae* s.s., *An. coluzzii,* and *An. arabiensis* were found in Simatou, Gounougou and Mangoum, while *An. gambiae* s.s. and *An. coluzzii* were recorded in Nyabessang. *An. coluzzii* was the only species of the *An. gambiae* complex recorded in Bonabéri (Figure 17). *An. coluzzii* constituted the main vector in Simatou (92.0%), Gounougou (91.6%), and Bonabéri (100%), in contrast to Mangoum and Nyabessang where *An. gambiae* s.s. was 99.3% and 96.7% of the population tested, respectively.

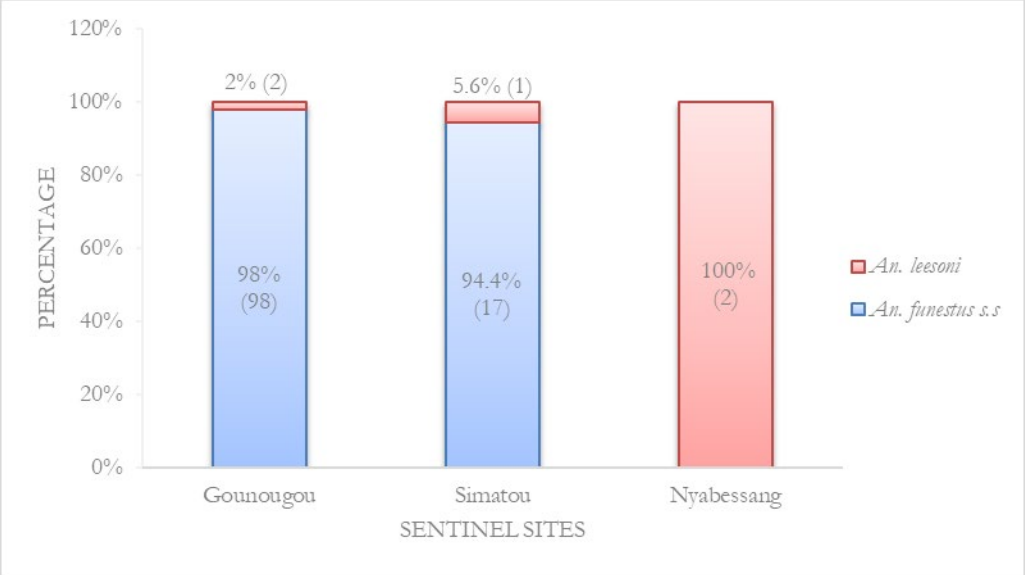
Figure 17: Species Composition of *An. gambiae* Complex Collected across All Sites



An. funestus Group

A total of 18 *An. funestus* s.l. from Simatou, 100 from Gounougou, and 2 from Nyabessang were molecularly identified. Two subspecies of the *An. funestus* group were found in Gounougou and Simatou: *An. funestus* s.s. (95.5%) and *An. leesoni* (4.5%). In Simatou and Gounougou, *An. funestus* s.s. was the most found; 97.8% in Gounougou and 94.4% in Simatou. *An. leesoni* was the only subspecies recorded in Nyabessang (Figure 18).

Figure 18: Species Composition of *An. funestus* Group of Simatou, Gounougou, and Nyabessang



3.1.3 Human Biting Rate and Seasonal Variation of *An. gambiae* s.l.

In the Northern sentinel sites, the average HBR was 38.2 bites/person/night (b/p/n) in Gounougou and 101.4 b/p/n in Simatou (Tables B1 and 2 in Annex B). The HBR varied monthly and the highest was observed in July in Simatou (327.2 b/p/n) and in March (86 b/p/n) in Gounougou (Figure 19).

In the Southern sentinel sites, the average HBR was 12.6 b/p/n in Bonabéri, 9.7 b/p/n in Mangoum, and 1.1 b/p/n in Nyabessang (Tables B3-B5 in Annex B). The highest HBR was recorded in July in Bonabéri (34.9 b/p/n), October in Mangoum (15.8 b/p/n), and Nyabessang (3.54 b/p/n) (Figure 20).

Figure 19: Human Biting Rate of *An. gambiae* s.l. in Gounougou and Simatou (November 2019-September 2020)

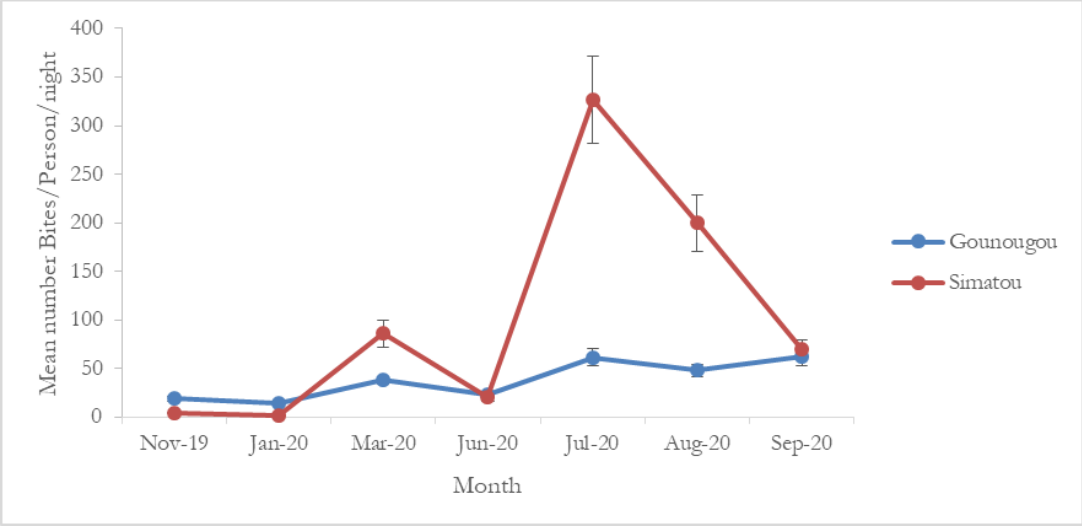
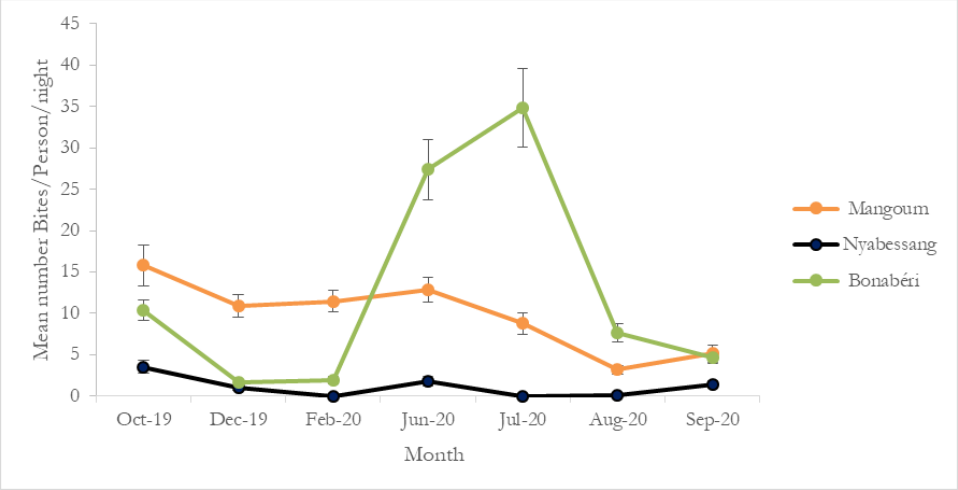


Figure 20: Human Biting Rate of *An. gambiae* s.l. in Mangoum, Nyabessang, and Bonabéri (October 2019-September 2020)



3.1.4 VECTOR BEHAVIOR

Biting Location of *An. gambiae* s.l.

In the northern sites of Gounougou and Simatou, *An. gambiae* s.l. biting was observed almost equally indoors and outdoors (Figures 21-22). Biting was slightly higher indoors than outdoors during the peak density periods in both sites. The endophagic index was 0.5 in Gounougou and 0.52 in Simatou (Tables B6-B7 in Annex B).

Figure 21: Indoor and Outdoor Biting of *An. gambiae* s.l. in Gounougou (November 2019-September 2020)

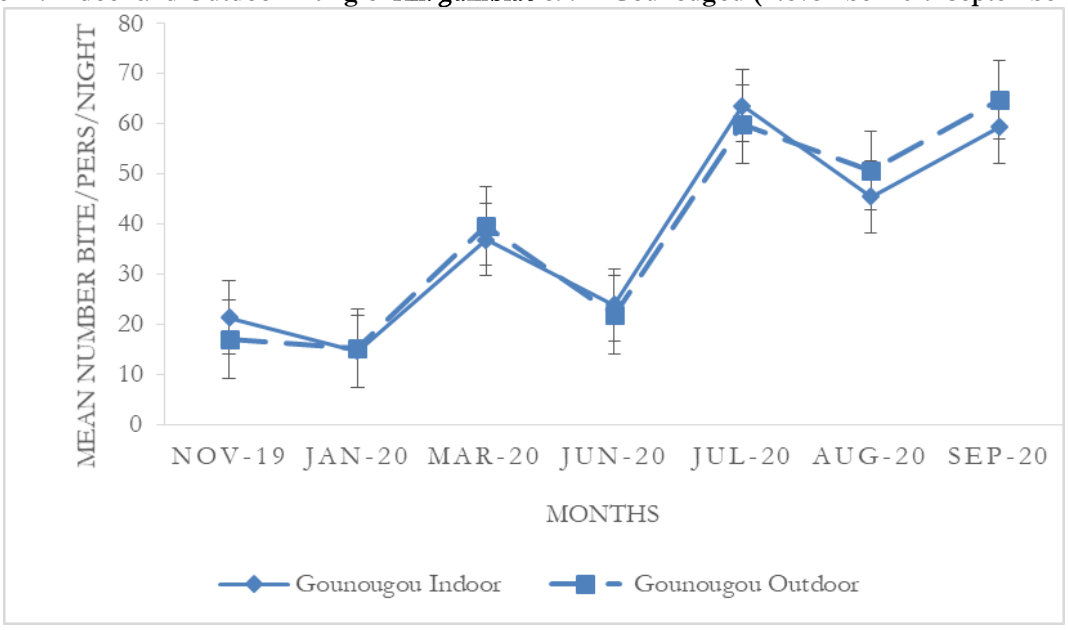
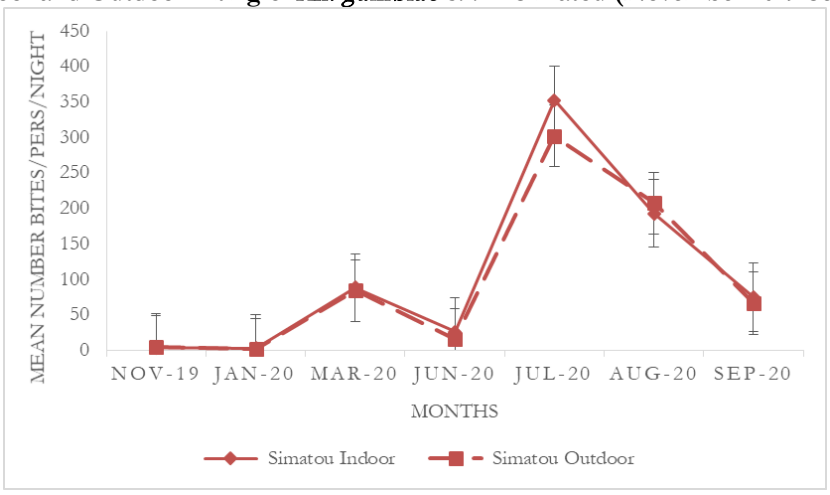


Figure 22: Indoor and Outdoor Biting of *An. gambiae* s.l. in Simatou (November 2019-September 2020)



In the three southern sites, biting by *An. gambiae* s.l. was higher outdoors than indoors throughout the year (Figures 23-25). The endophagic index was 0.46 in Mangoum, 0.43 in Nyabessang, and 0.35 in Bonabéri (Tables B8-B10, Annex B).

Figure 23: Indoor and Outdoor Biting of *An. gambiae* s.l. in Mangoum (October 2019-September 2020)

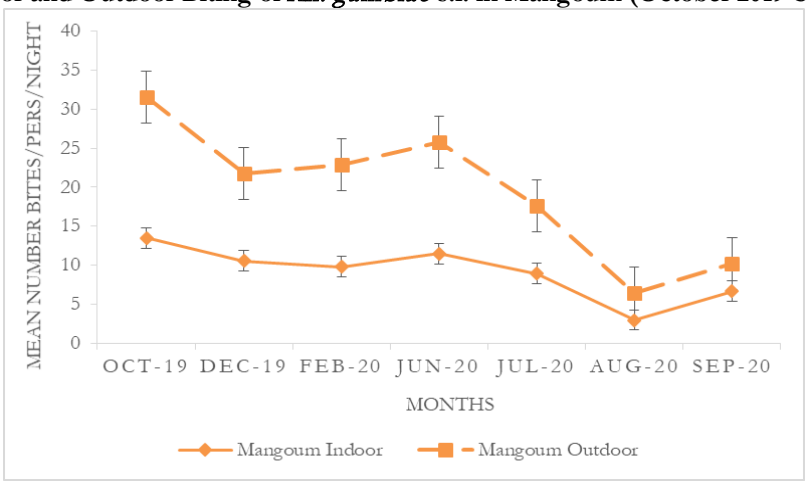


Figure 24: Indoor and Outdoor Biting of *An. gambiae* s.l. in Nyabessang (October 2019-September 2020)

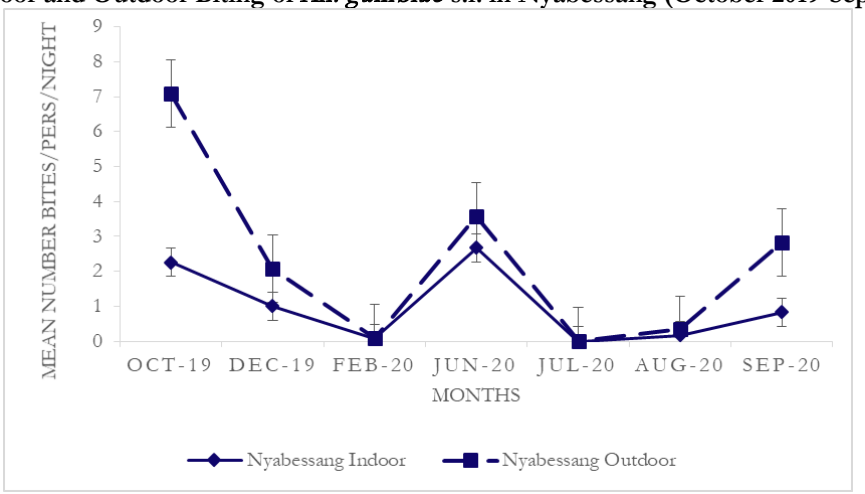
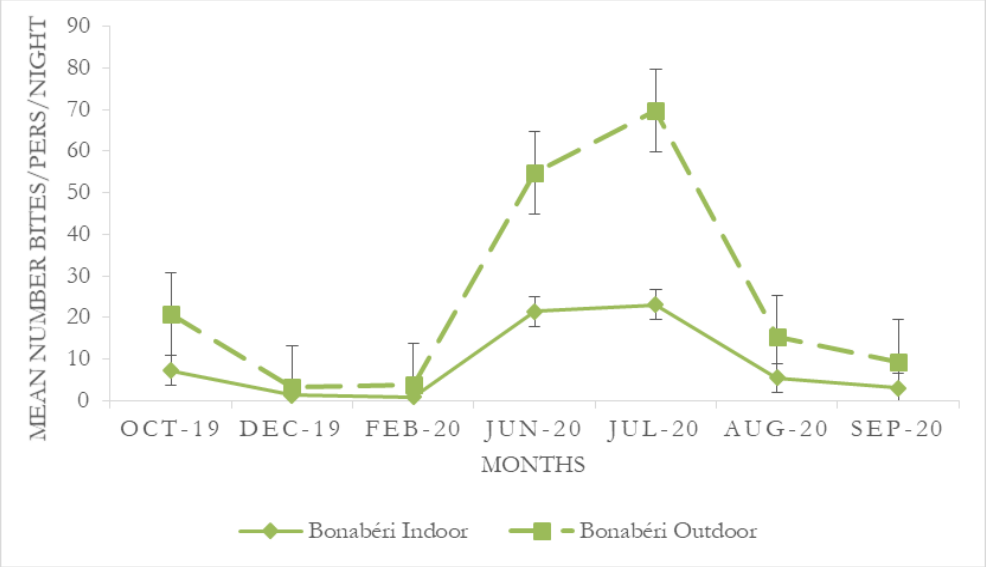


Figure 25: Indoor and Outdoor Biting of *An. gambiae* s.l. in Bonabéri (October 2019-September 2020)



Hourly Biting Time of *An. gambiae* s.l.

In all sites, biting peaked both indoors and outdoors after midnight (Figure 26-27), except in Simatou where the peak densities started at 11:00 p.m., both indoors and outdoors. The densities of *An. gambiae* s.l. were fairly stable throughout the night after midnight. Simatou recorded the highest hourly biting peaks: about 10.9 bites/person/hour (b/p/h) were recorded between 4:00-5:00 a.m. indoors and about 11.4 b/p/h were recorded between 5:00-6:00 a.m. outdoors). Biting continued indoors and outdoors between 7:00 a.m. and 8:00 a.m. in Gounougou and Simatou particularly, where HBRs were 2.4 b/p/h and 4.6 b/p/h, respectively (Figures 26-27). In general, lower biting rates were recorded in the three Southern sites, with an average of 1 b/p/h throughout the night and less than 0.2 b/p/h recorded both indoors and outdoors after 7:00 a.m. (Figures 26-27).

Figure 26: Indoor Hourly Biting of *An. gambiae* s.l. across Sites

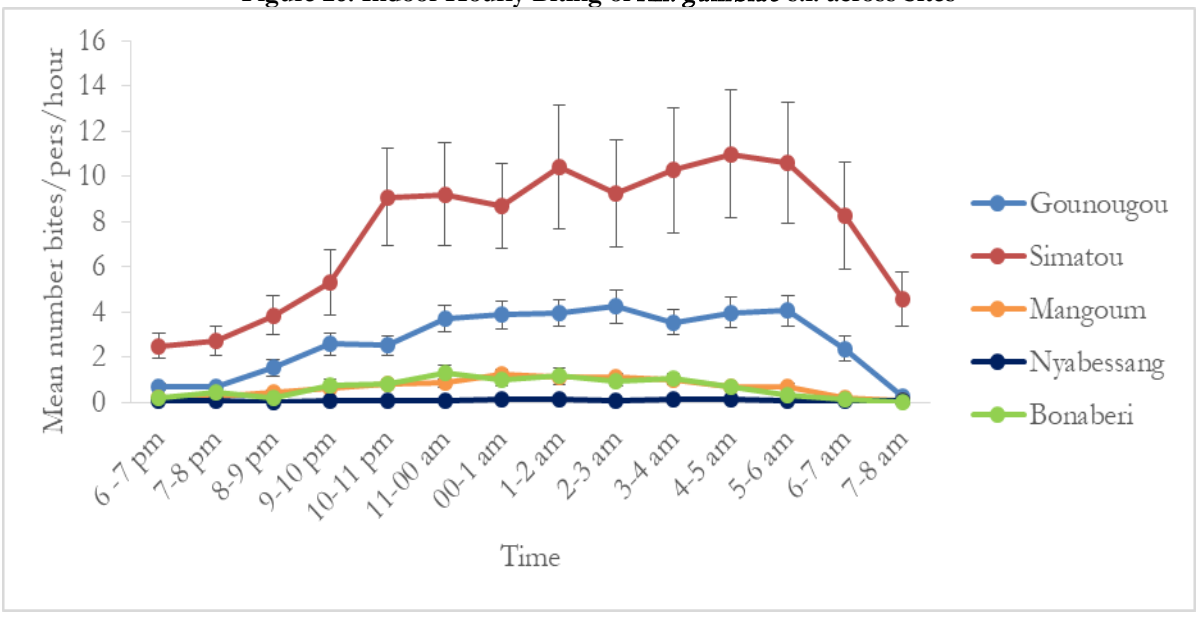
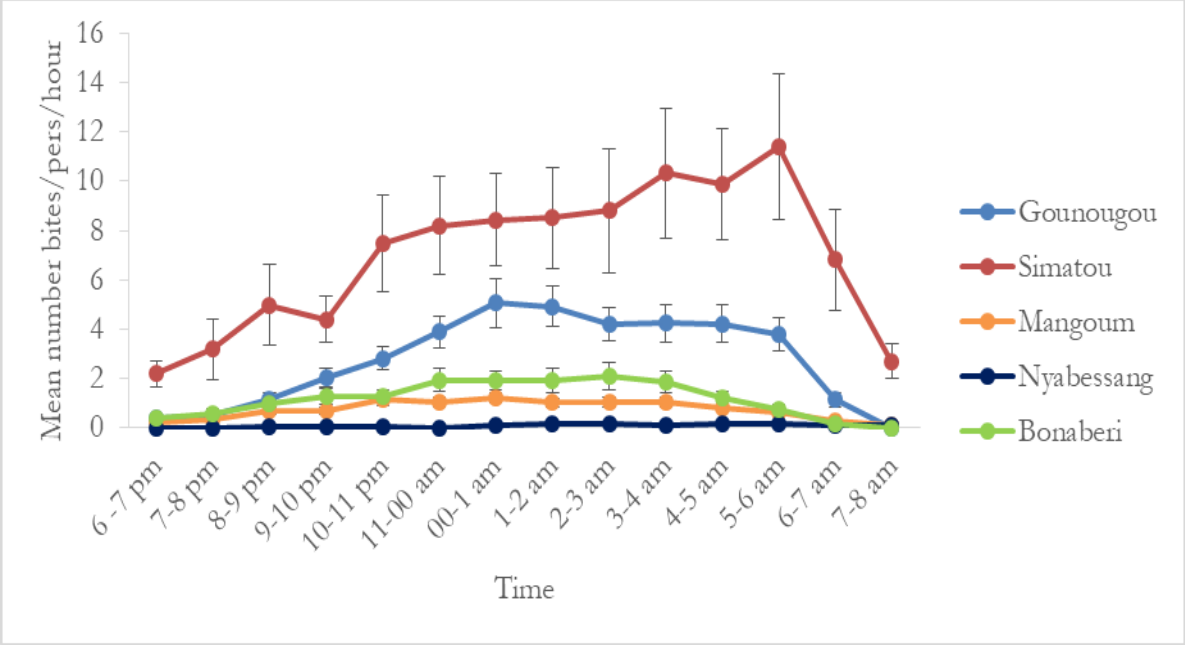


Figure 27: Outdoor Hourly Biting of *An. gambiae* s.l. across Sites



3.1.5 Human Biting Rate and Seasonal Variation of Other Vectors

As described above, each site recorded additional specific *Anopheles* vector species besides *An. gambiae* s.l., except in Bonabéri.

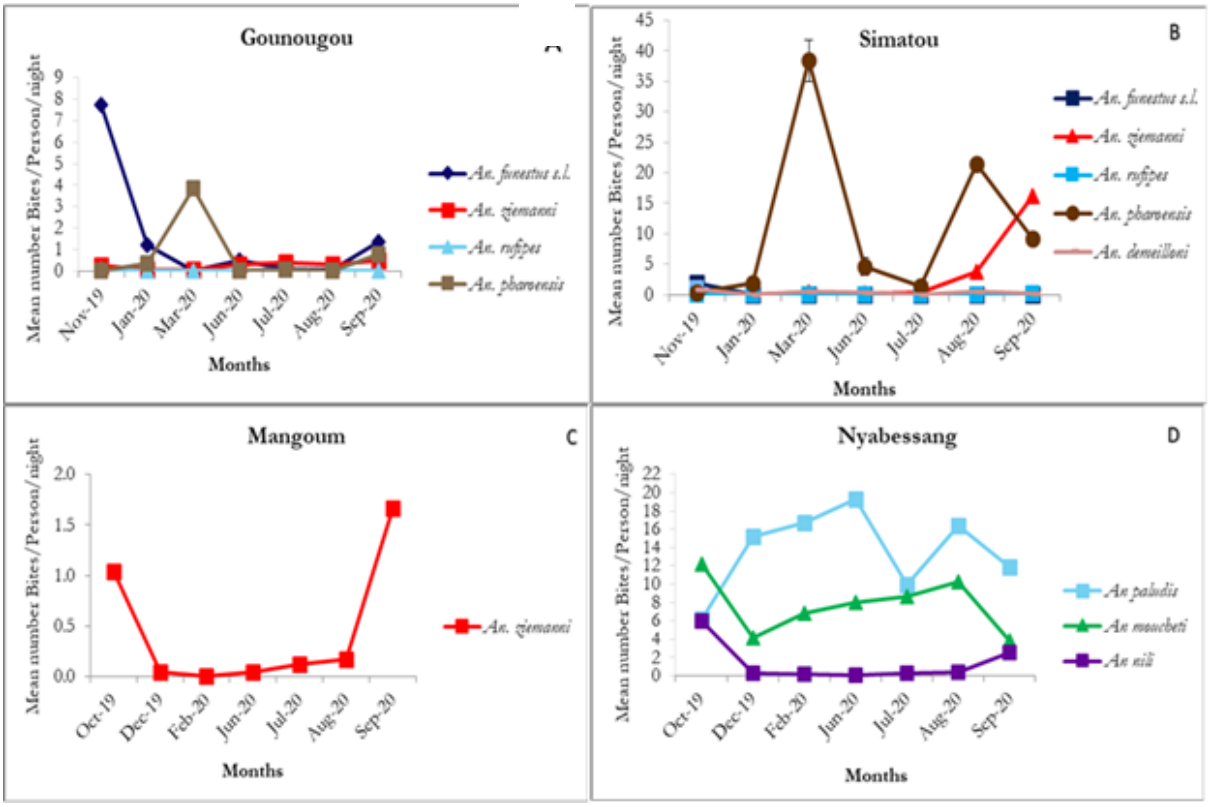
In Gounougou, *An. funestus* s.l. and *An. pharoensis* were the most dominant though *An. rufipes* and *An. ziemanni* were also found during all seven months of collections (Figure 28A and Table A3 in Annex A). The highest HBR of *An. funestus* s.l. was recorded in November 2019 (7.8 b/p/n), while *An. pharoensis* peaked in March 2020 (3.9 b/p/n).

In Simatou, *An. funestus, An. ziemanni,* and *An. pharoensis* were the three major *Anopheles* species found in addition to *An. gambiae* s.l. *An. pharoensis* recorded the highest density in Simatou compared to all the other four sites with two peaks recorded in March 2020 (38.4 b/p/n) and in August 2020 (21.5 b/p/n). *An. funestus* s.l. was collected mostly between November 2019 and January 2020 while *An. ziemanni* was found from July to September 2020 with the highest peak in September (16.3 b/p/n) (Figure 28B).

An. ziemanni represented the only secondary *Anopheles* species in Mangoum and few numbers were collected. The average HBR of *An. ziemanni* was 1 b/p/n with a peak in October 2019 (1 b/p/n) and September 2020 (1.7 b/p/n) (Figure 28C).

In Nyabessang, five *Anopheles* species were found with *An. moucheti* and *An. paludis* being the predominant species in addition to *An. nili* (Table A3). *An. moucheti* and *An. paludis* were collected during all collection months; *An. moucheti* peaked in October 2019 with 12.2 b/p/n, while *An. paludis* peaked in June 2020 with 19.3 b/p/n (Figure 28D).

Figure 28: Human Biting Rates and Seasonal Variation of Other *Anopheles* Vectors by Site (October 2019-September 2020)



3.1.6 INDOOR RESTING DENSITY OF *AN. GAMBIAE* S.L.

Across sites, the average *Anopheles* density per room was 35.1 females/room/night (9,808 total females/280 room-nights). Figures 29 to 30 illustrate the trend in the Northern and Southern sites for *An. gambiae* s.l., the main vector collected in all the sites from October 2019 to September 2020.

Figure 29: Indoor Resting Density of *An. gambiae* s.l. in Gounougou and Simatou (November 2019-September 2020)

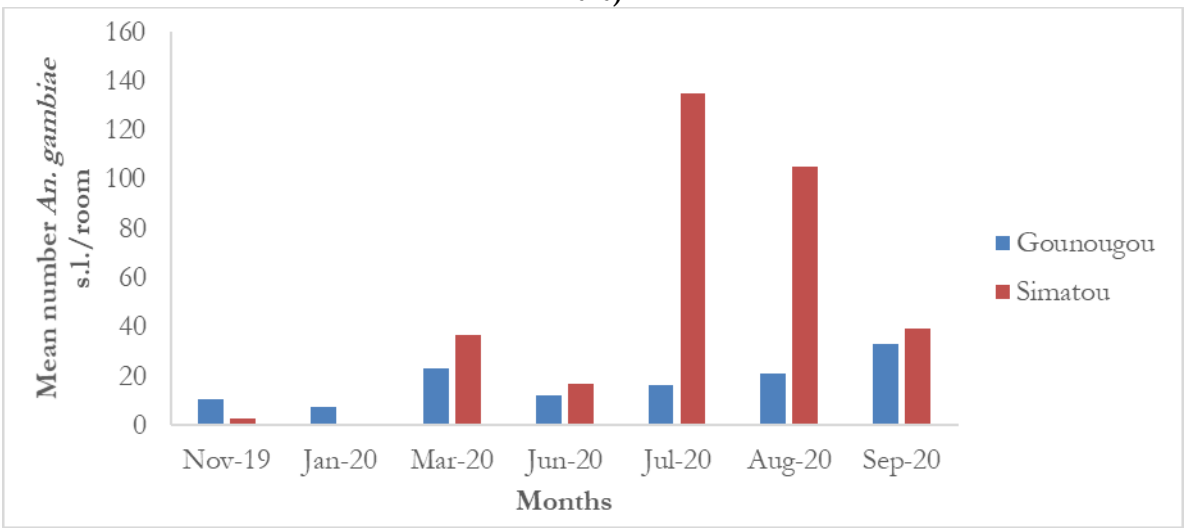
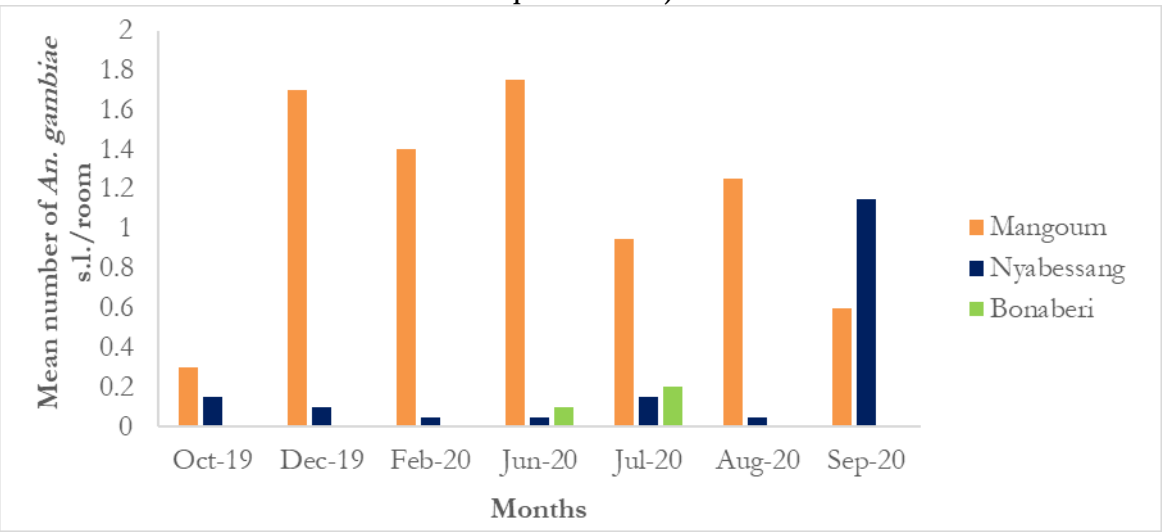


Figure 30: Indoor Resting Density of *An. gambiae* s.l. in Mangoum, Nyabessang, and Bonabéri (October 2019-September 2020)



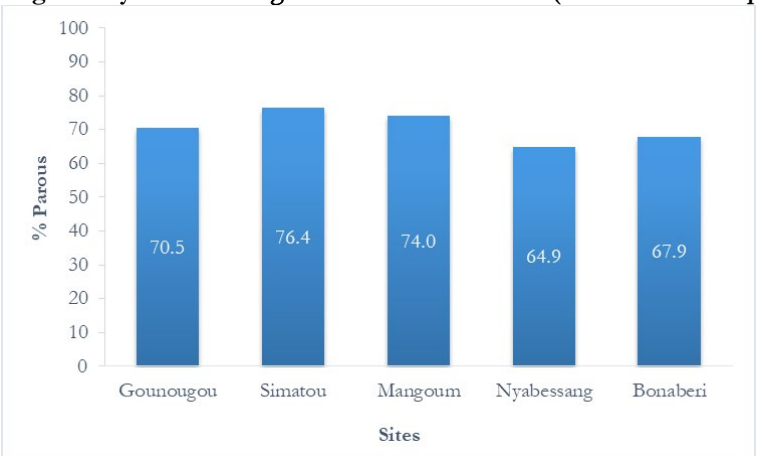
3.1.7 HOST PREFERENCE

A total of 1,407 blood-fed *Anopheles* mosquitoes collected by PSCs (726 from Gounougou, 595 from Simatou, 67 from Mangoum, 17 from Nyabessang, and 2 from Bonabéri) were analyzed using ELISA, of which 719 were found to have fed on humans. The average HBI was 51.1% (Table C1 in Annex C) and varied from 47.0% in Simatou to 94.0% in Mangoum.

3.1.8 PARITY

The ovaries of 5,760 *Anopheles* mosquitoes were dissected. The average parity rate across the five sites was 72.1%. For *An. gambiae* s.l., high parity rate was observed in all sites ranging from 64.9% in Nyabessang to 76.4% in Simatou (Figure 31) (Table C2 in Annex C).

Figure 31: Average Parity Rate of *An. gambiae* s.l. across Sites (October 2019–September 2020)



3.1.9 ENTOMOLOGICAL INOCULATION RATE PER SITE

A total of 6,764 *Anopheles* mosquitoes were tested by ELISA and 106 were detected with circumsporozoite antigen of *Plasmodium falciparum*. The total average infection rate was 1.56% (Table C3 in Annex C). Ten *Anopheles* species were found to be positive: *An. gambiae* s.l., *An. funestus* s.l., *An. nili*, *An. moucheti*, *An. demeillonni*, *An. pharoensis*, *An. ziemanni*, *An. multicinctus, An. rufipes*, and *An. marshallii*. The infection rates by site were:

Gounougou (1.42%), Simatou (0.66%), Mangoum (2.85%), Nyabessang (1.86%), and Bonabéri (1.61%). The mean EIR varied from 6.1 infected bites/person/month (ib/p/m) in Bonabéri to 22.8 ib/p/m in Simatou (Table 3).

Table 3: EIR of *Anopheles* Mosquitoes Collected per Site by HLC (October 2019-September 2020)

Sentinel Site	Species	HBR	Infection Rate	EIR (ib/p/n)	Monthly EIR (ib/p/m)
Gounougou	*An. gambiae* s.l.	38.2	0.012	0.458	13.75
	An. funestus s.l.	1.6	0.016	0.02	0.77
	An. multicinctus	0.2	0.03	0.01	0.20
	An. ziemanni	0.3	0.07	0.022	0.66
Total EIR		**40.25**	**0.014**	**0.58**	**17.4**
Simatou	*An. gambiae* s.l.	101.5	0.0043	0.443	13.30
	An. funestus s.l.	0.3	0.07	0.02	0.64
	An. rufipes	0.01	0.5	0.005	0.15
	An. pharoensis	11.1	0.008	0.088	2.66
Total EIR		**112.88**	**0.007**	**0.759**	**22.8**
Mangoum	*An. gambiae* s.l.	9.7	0.028	0.273	8.20
	An. ziemanni	0.4	0.013	0.005	0.162
Total EIR		**10.17**	**0.028**	**0.29**	**8.70**
Nyabessang	*An. gambiae* s.l.	1.1	0.075	0.082	2.48
	An. moucheti	7.7	0.008	0.07	2.04
	An. nili	1.4	0.04	0.053	1.59
	An. paludis	13.7	0.010	0.146	4.38
	An. marshallii	0.7	0.015	0.010	0.32
Total EIR		**24.55**	**0.017**	**0.426**	**12.78**
Bonabéri	*An. gambiae* s.l.	12.7	0.016	0.205	6.16
	Total EIR	**12.7**	**0.016**	**0.205**	**6.16**

3.2 INSECTICIDE RESISTANCE MONITORING

3.2.1 SUSCEPTIBILITY STATUS OF *AN. GAMBIAE* S.L.

Anopheles gambiae s.l. from five sites were surveyed (Gounougou, Simatou, Nyabessang, Mangoum, and Bonabéri) in September 2020. Figures 32–36 below show the resistance status of *An. gambiae* s.l. to the different pyrethroids, carbamate, and organophosphate classes of insecticide tested at each site (Annex D1).

Resistance was observed to the diagnostic dose of all pyrethroids in all sites. Resistance to pirimiphos-methyl and bendiocarb was observed in Mangoum and susceptibility in Gounougou, Simatou, and Bonabéri. In Nyabessang, *An. gambiae* s.l. was resistant to bendiocarb and susceptible to pirimiphos-methyl.

High pyrethroid resistance intensity (less than 98% mortality at 10x the diagnostic dose) to deltamethrin, permethrin, and alpha-cypermethrin was observed in Gounougou, Simatou, Nyabessang, and Mangoum. Moderate resistance (below 98% mortality at 5x or greater than 98% at 10x the diagnostic dose) was observed at Bonabéri for permethrin, deltamethrin, and alpha-cypermethrin (Figure 36).

Note: The red and green lines in Figures 32-39 represent the resistance and susceptibility thresholds, respectively.

Figure 32: WHO Susceptibility Test Results for *An. gambiae* s.l. in Gounougou

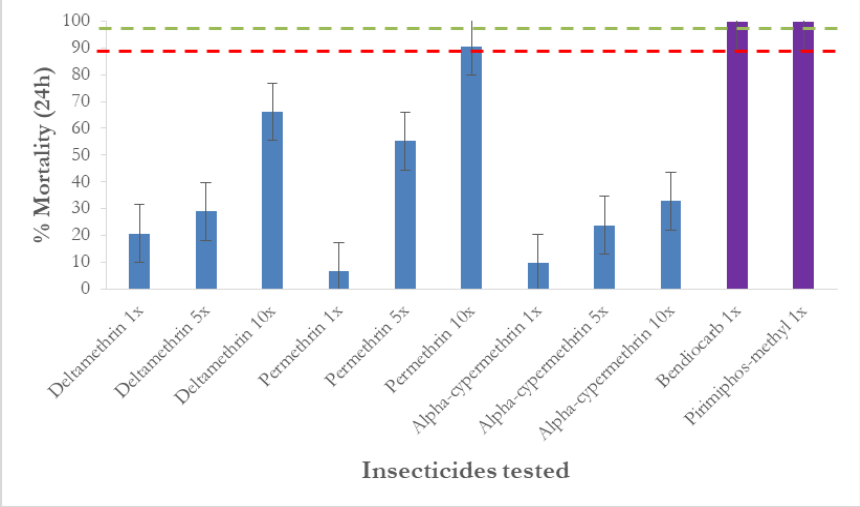


Figure 33: WHO Susceptibility Test Results for *An. gambiae* s.l. in Simatou

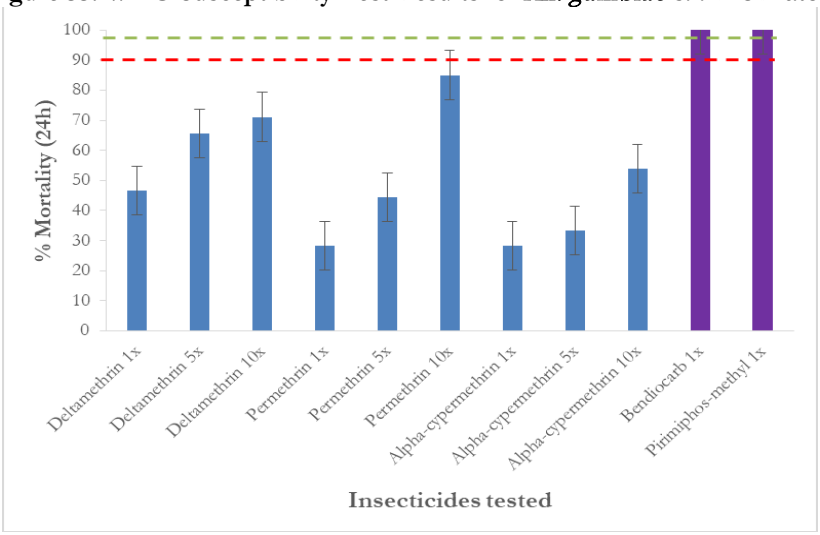


Figure 34: WHO Susceptibility Test Results for *An. gambiae* s.l. in Mangoum

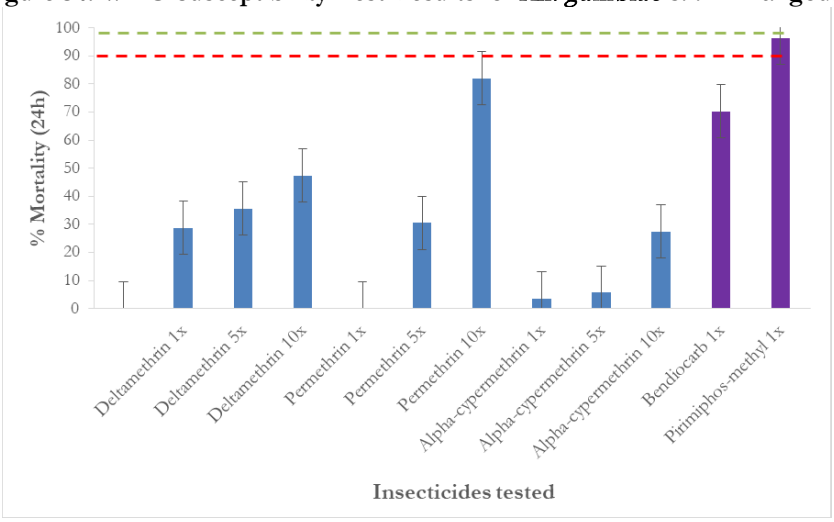


Figure 35: WHO Susceptibility Test Results for *An. gambiae* s.l. in Nyabessang

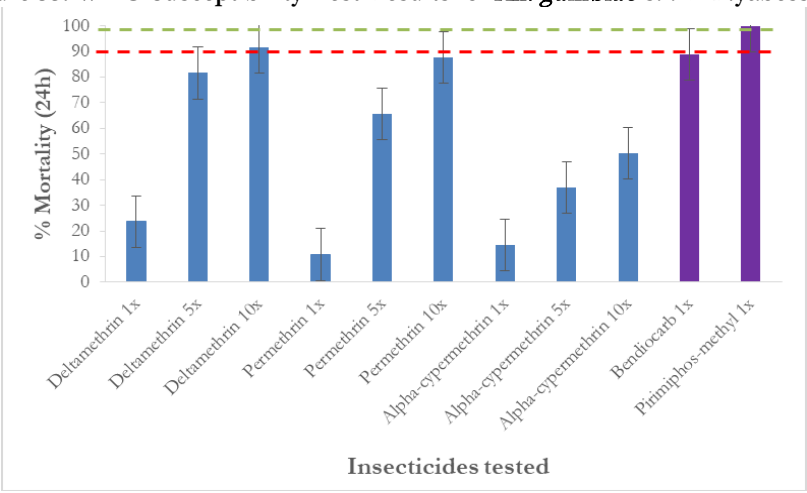
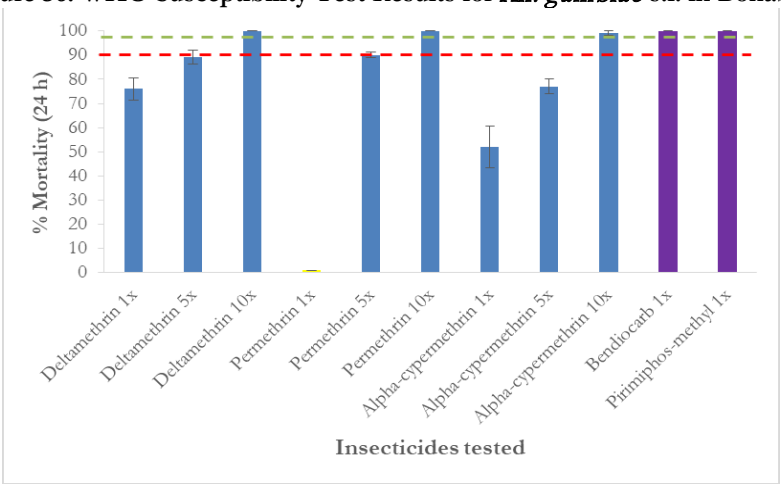
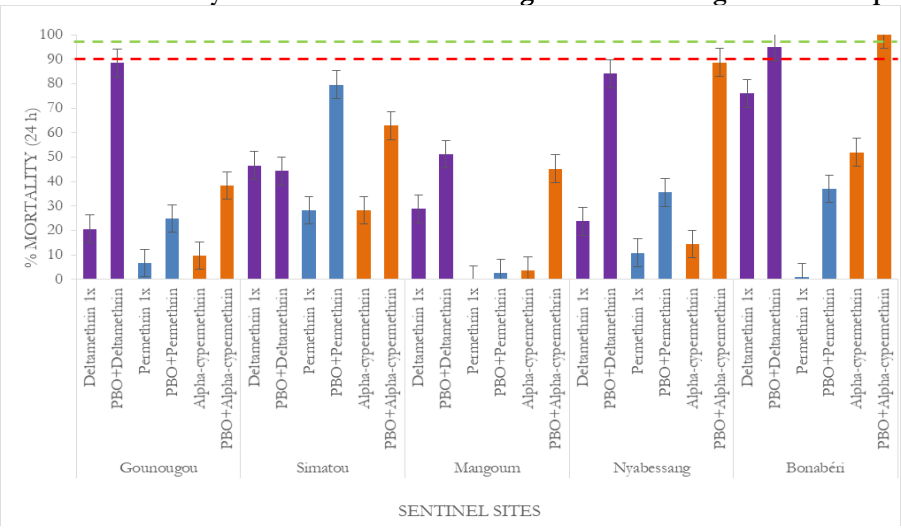


Figure 36: WHO Susceptibility Test Results for *An. gambiae* s.l. in Bonabéri



Pre-exposure of mosquitoes to PBO before deltamethrin, permethrin, or alpha-cypermethrin partially increased the mortality of *An. gambiae* s.l. but did not reach full susceptibility in all sites surveyed except in Bonabéri with alpha-cypermethrin.

Figure 37: Effect of PBO on Pyrethroid Resistance of *An. gambiae* s.l. using WHO Susceptibility Tests



Susceptibility to clothianidin was recorded after 48 hours in Nyabessang and Bonabéri and after 72 hours in Simatou and Gounougou. In Mangoum, susceptibility was recorded after seven days (Figure 38). Susceptibility to chlorfenapyr (100 µg/bottle) was recorded 24 hours post-exposure in Nyabessang and 72 hours post-exposure in Simatou and Bonabéri. Susceptibility to chlorfenapyr (100 µg/bottle) was observed in all five sites after 72 hours (Figure 39 and Tables D2-D3 in Annex D).

Figure 38: Susceptibility of *An. gambiae* s.l. to Clothianidin 2% using WHO Susceptibility Test at All Sites

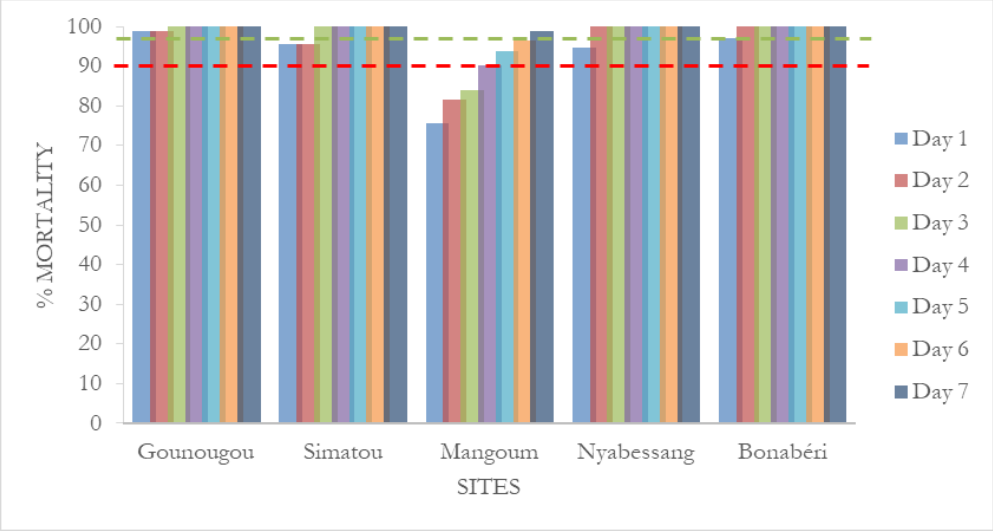
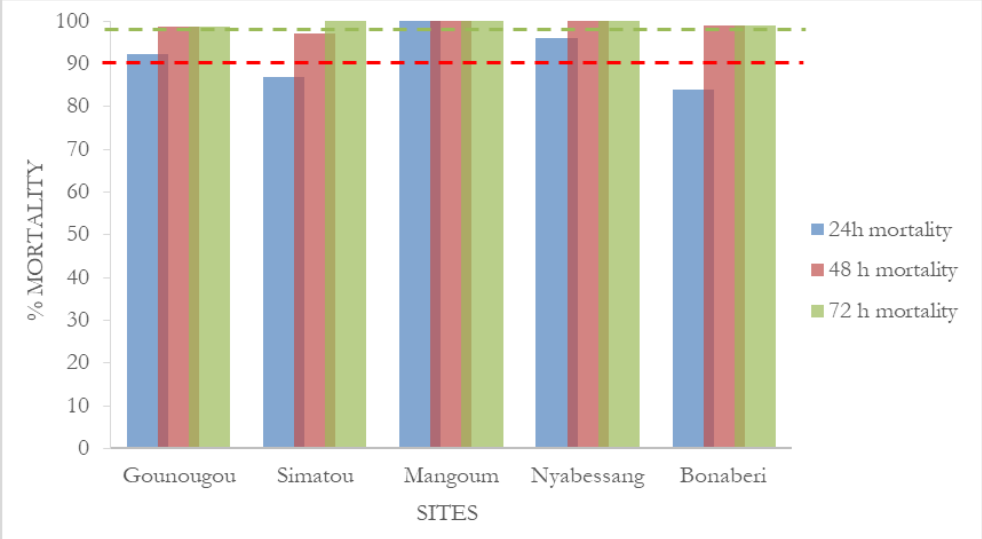


Figure 39: Susceptibility of *An. gambiae* s.l. to Chlorfenapyr (100 µg/bottle) using CDC Bottle Assay at All Sites



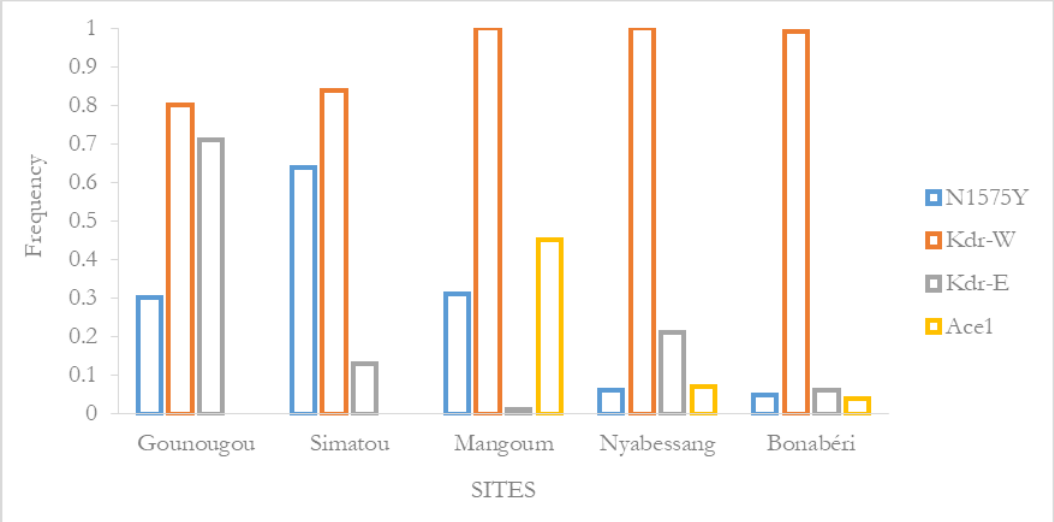
3.2.2 Insecticide Resistance Mechanisms

3.2.2.1 Target Site Resistance

Insecticide resistance in mosquitoes can be related to target site mutations. Among them, resistance to pyrethroids and DDT is described as a substitution of amino acid leucine to either phenylalanine (L1014F, referred as *kdr*-West) or serine (L1014S, referred as *kdr*-East) at the position 1014 in the sodium channel gate. The N1575Y represents and additional mutation involved in the *kdr* mutation. For organophosphate and carbamate insecticide, target site mechanism, known as *ace-1* (G296S) is a substitution of an amino acid Glycine to Serine at position 119. Four mutations were detected in the *An. gambiae* s.l. populations of the Southern sites

(Mangoum, Nyabessang, and Bonabéri): *Kdr*-w (L1014F), *Kdr*-e (L1014S), N1575Y for pyrethroids, and *Ace*-1 for organophosphates and carbamates. The *Ace*-1 mutation was not found in Gounougou and Simatou. Furthermore, the *Kdr*-w mutation was highly present in all sites and already had been observed previously in Mangoum, Nyabessang, and Bonabéri (100% resistance allele). Gounougou and Simatou recorded a frequency of 80% and 84%, respectively. In contrast, the *Kdr*-e mutation was mostly found in Gounougou (71%) compared to the other four sites (Figure 40 and Table D4 in Annex D). Also, the N1575Y mutation was detected in all sites, with the highest frequency recorded in Simatou (64%) followed by Gounougou and Mangoum, each with about 30% resistance allele frequency.

Figure 40: Frequency of Target Site Mechanisms Involved in Insecticide Resistance of *An. gambiae* s.l.



4. Discussion and Conclusions

Longitudinal vector surveillance was conducted from October 2019 to March 2020 and from June to September 2020 in five sentinel sites of Cameroon. April and May collection could not be undertaken due to the COVID-19 pandemic. The data collected showed a large diversity of *Anopheles* species across sites, similar to the past two years. Overall, 12 *Anopheles* species were recorded in the sites and *An. gambiae* s.l., was the most abundant vector collected, and was found in all sites and through all collection methods. The composition of species across the sites was similar as 2018 and 2019. *An. moucheti* and *An. nili* were collected only in Nyabessang. *An. gambiae* s.l. was collected in all sites at variable proportions depending on the collection method. In Bonabéri, *An. gambiae* s.l., specifically *An. coluzzii*, represented the only vector collected using all three collection methods, while *An. ziemanni* was the only additional *Anopheles* species (besides *An. gambiae* s.l.) found in Mangoum and was collected using both HLCs and CDC LTs. *An. moucheti* and *An. paludis* were collected through all collection methods only in Nyabessang; *An. nili* from Nyabessang was found using HLCs and CDC LTs.

Three sub-species of *An. gambiae* complex (*An. gambiae* s.s., *An. coluzzii*, and *An. arabiensis)* were identified. *An. arabiensis* was found in the drier areas (Gounougou and Simatou). *An. leesoni* and *An. funestus* s.s. were also found in Gounougou and in Simatou. Ten *Anopheles* species out of the 12 identified were found to be involved in malaria transmission per the detection of sporozoite parasite after ELISA tests. The monthly entomological inoculation rate varied from 6.1 infected b/p/m in Bonabéri to 22.8 infected b/p/m in Simatou.

Continuous biting by vectors was observed during the early morning until 8:00 am, particularly in the northern sites of Gounougou and Simatou. These results suggest the importance of conducting an ecological survey to understand the eco-biology of these *Anopheles* species in the country for appropriate control measures, as they are in majority involved in malaria transmission.

Overall, similar biting patterns, species composition, and behavior during this reporting period were observed as the previous 2018-2019 report. The peak biting time for *An. gambiae* was the same in each of the sites (between June and August), while the peak biting hour still occurred after midnight.

As reported in the 2018/2019 report, resistance to all three pyrethroids tested (deltamethrin, permethrin, and alpha-cypermethrin) was observed in all sites. The intensity of the resistance varied according to the sites and the insecticides. High resistance intensity was recorded in Gounougou, Simatou, and Mangoum for all three pyrethroids while moderate intensity resistance was observed in Nyabessang and Bonabéri for permethrin and alpha-cypermethrin. Pre-exposure to PBO partially restored susceptibility to all three pyrethroids at all sites, except in Bonabéri where full restoration of susceptibility was only observed with PBO + alpha-cypermethrin. Furthermore, at all sites, PBO + deltamethrin (33.5% average increase) and PBO + alpha-cypermethrin (45.4% average increase) increased the mortality more than PBO + permethrin (26.6% average increase) at all sites. This indicates that PBO ITNs, particularly with deltamethrin or alpha-cypermethrin, could be considered for ITN procurement and distribution in sites with substantial increase in mortality after pre-exposure of the vectors to PBO. PBO + deltamethrin ITNs could be suitable for Gounougou, Nyabessang, and Bonabéri, while PBO + alpha-cypermethrin ITNs could be distributed in Simatou, Nyabessang, Mangoum, and Bonabéri.

Susceptibility to chlorfenapyr (100 µg/bottle) was also recorded in all five sites, suggesting that chlorfenapyr-treated ITNs, such as the Interceptor G2, could be an appropriate option in these high pyrethroid resistance sites.

In 2020, *An. gambiae* s.l. was susceptible to pirimiphos-methyl in four of the five sites, while probable resistance was observed in Mangoum. In 2019, all sites were susceptible to pirimiphos-methyl. However, *An. gambiae* s.l. was susceptible to bendiocarb in only three of the sites (Simatou, Gounougou, and Bonabéri). Susceptibility to clothianidin 2% was recorded at all sites, suggesting that new interventions may be promising in Cameroon.

Nevertheless, in the rural and urban ecosystems around Yaoundé in Cameroon, resistance of *An. gambiae* s.l. to clothianidin was found (Fouet et *al.*, 2020). As in 2018 and 2019, two types of resistance mechanisms—target site (*Kdr*, *Ace*-1*,* and *N1575Y*) and metabolic—were found indicating that multiple mechanisms are involved in the insecticide resistance of the malaria vectors, which require appropriate management strategies.

These results of the entomological monitoring will support the NMCP for vector control and strategy decision making as the country reviews its resistance management plan for the next five years and plans the targeted distribution on PBO and dual AI ITNs in the country. The biting and resting behavior of the vectors, coupled with the diversity of vectors capable to transmit the sporozoite, and the high level of resistance to insecticides used for ITNs suggests an additional vector control strategy such as indoor residual spraying may be needed to reduce the malaria transmission and burden in the country.

ANNEX A: SPECIES COMPOSITION OF *ANOPHELES* BY METHOD AND SITE

Table A1: *Anopheles* Species Collected by HLCs by Site (October 2019-September 2020)

Anopheles species	Simatou	Nyabessang	Mangoum	Gounougou	Bonabéri	Total
An. gambiae s.l.	17,050	192	1,635	6,416	2,126	**27,419**
An. paludis	0	2,294	0	0	0	**2,294**
An. pharoensis	1,860	0	0	122	0	**1,982**
An. moucheti	0	1,294	0	0	0	**1,294**
An. ziemanni	505	0	74	45	0	**624**
An. funestus	48	0	0	264	0	**312**
An. nili	0	227	0	0	0	**227**
An. marshallii	0	119	0	0	0	**119**
An. demeilloni	64	0	0	0	0	**64**
An. multicinctus	0	0	0	38	0	**38**
An. rufipes	6	0	0	2	0	**8**
An. welcomei	1	0	0	0	0	**1**
Total	**19,534**	**4,126**	**1,709**	**6,887**	**2,126**	**34,382**

Table A2: *Anopheles* Species Collected by CDC LTs by Site (October 2019-September 2020)

Anopheles species	Simatou	Nyabessang	Mangoum	Gounougou	Bonabéri	Total
An. gambiae s.l.	6,715	52	402	2,177	14	**9,360**
An. ziemanni	777	0	3	108	0	**888**
An. pharoensis	345	0	0	11	0	**356**
An. demeilloni	140	0	0	0	0	**140**
An. paludis	0	106	0	0	0	**106**
An. funestus s.l.	46	0	0	54	0	**100**
An. rufipes	80	0	0	7	0	**87**
An. moucheti	0	65	0	0	0	**65**
An. multicinctus	0	0	0	10	0	**10**
An. nili	0	7	0	0	0	**7**
An. marshallii	0	2	0	0	0	**2**
Total	**8,103**	**232**	**405**	**2,367**	**14**	**11,121**

Table A3: *Anopheles* Species Collected by PSC by Site (October 2019-September 2020)

Anopheles species	Simatou	Nyabessang	Mangoum	Gounougou	Bonabéri	Total
An. gambiae s.l.	6,750	34	159	2,483	6	**9,432**
An. funestus s.l.	37	0	0	129	0	**166**
An. rufipes	125	0	0	2	0	**127**
An. demeilloni	39	0	0	0	0	**39**
An. pharoensis	30	0	0	0	0	**30**
An. ziemanni	6	0	0	0	0	**6**
An. paludis	0	5	0	0	0	**5**
An. moucheti	0	3	0	0	0	**3**
An. multicinctus	0	0	0	1	0	**1**
Total	**6,987**	**42**	**159**	**2,615**	**6**	**9,809**

Table A4: Species Composition of *An. gambiae* s.l. and *An. funestus* s.l. by Site (October 2019-September 2020)

	An. gambiae s.l.					*An. funestus* s.l.		
Sites	*An. gambiae* s.s.	*An. arabiensis*	*An. coluzzii*	*An. coluzzii/ An. gambiae*	**Total**	*An. funestus* s.s.	*An. leesoni*	**Total**
Gounougou	9	61	761	0	**831**	88	2	**90**
Simatou	2	67	797	0	**866**	17	1	**18**
Mangoum	548	1	2	1	**552**	0	0	**0**
Nyabessang	177	0	6	0	**183**	0	2	**2**
Bonabéri	0	0	347	0	**347**	0	0	**0**
Total	**736**	**129**	**1,913**	**1**	**2,779**	**105**	**5**	**110**

ANNEX B: HUMAN BITING RATE OF *ANOPHELES* MOSQUITOES SPECIES BY SITE

Table B1: Human Biting Rate of *Anopheles* Mosquitoes from Simatou using HLCs (November 2019-September 2020)

Anopheles species	Nov-19		Jan-20		Mar-20		Jun-20		Jul-20		Aug-20		Sep-20		Total	
	TC	HBR	TC	HBR	TC	HBR	TC	HBR	TC	HBR	TC	HBR	TC	HBR	TC	HBR
An. gambiae s.l.	462	19.25	358	14.92	920	38.33	550	22.92	1,483	61.79	1,154	48.08	1,489	62.04	6,416	38.19
An. ziemanni	6	0.25	2	0.08	2	0.08	7	0.29	9	0.38	7	0.29	12	0.50	45	0.27
An. funestus s.l.	186	7.75	29	1.21	0	0.00	12	0.50	3	0.13	2	0.08	32	1.33	264	1.57
An. pharoensis	0	0.00	8	0.33	93	3.88	0	0.00	2	0.08	0	0.00	19	0.79	122	0.73
An. rufipes	1	0.04	0	0.00	0	0.00	0	0.00	1	0.04	0	0.00	0	0.00	2	0.01
An. multicinctus	3	0.13	27	1.13	8	0.33	0	0.00	0	0.00	0	0.00	0	0.00	38	0.23

Table B2: Human Biting Rate of *Anopheles* Mosquitoes from Gounougou using HLCs (November 2019-September 2020)

Anopheles species	Nov-19		Jan-20		Mar-20		Jun-20		Jul-20		Aug-20		Sep-20		Total	
	TC	HBR	TC	HBR	TC	HBR	TC	HBR	TC	HBR	TC	HBR	TC	HBR	TC	HBR
An. gambiae s.l.	102	4.25	44	1.83	2,066	86.08	491	20.46	7,852	327.17	4,802	200.08	1,693	70.54	**17,050**	**101.49**
An. ziemanni	1	0.04	0	0.00	8	0.33	6	0.25	10	0.42	90	3.75	390	16.25	**505**	**3.01**
An. funestus s.l.	45	1.88	2	0.08	0	0.00	0	0.00	0	0.00	0	0.00	1	0.04	**48**	**0.29**
An. pharoensis	10	0.42	45	1.88	921	38.38	112	4.67	34	1.42	516	21.50	222	9.25	**1,860**	**11.07**
An. rufipes	2	0.08	0	0.00	1	0.04	0	0.00	0	0.00	0	0.00	3	0.13	**6**	**0.04**
An. welcomei	1	0.04	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	**1**	**0.01**
An. demeilloni	23	0.96	0	0.00	14	0.58	10	0.42	0	0.00	14	0.58	3	0.13	**64**	**0.38**

Table B3: Human Biting Rate of *Anopheles* Mosquitoes from Mangoum using HLCs (October 2019-September 2020)

Anopheles species	Oct-19		Dec-19		Feb-20		Jun-20		Jul-20		Aug-20		Sep-20		Total	
	TC	HBR	TC	HBR	TC	HBR	TC	HBR	TC	HBR	TC	HBR	TC	HBR	TC	HBR
An. gambiae s.l.	379	15.79	261	10.88	275	11.46	309	12.88	211	8.79	77	3.21	123	5.13	**1,635**	**9.73**
An. ziemanni	25	1.04	1	0.04	0	0.00	1	0.04	3	0.13	4	0.17	40	1.67	**74**	**0.44**

TC=Total Collected, HBR=Human Biting Rate

Table B4: Human Biting Rate of *Anopheles* Mosquitoes from Nyabessang using HLCs (October 2019-September 2020)

Anopheles species	Oct-19		Dec-19		Feb-20		Jun-20		Jul-20		Aug-20		Sep-20		Total	
	TC	HBR	TC	HBR	TC	HBR	TC	HBR	TC	HBR	TC	HBR	TC	HBR	TC	HBR
An. gambiae s.l.	85	3.54	25	1.04	1	0.04	43	1.79	0	0.00	4	0.17	34	1.42	**192**	**1.14**
An. paludis	149	6.21	365	15.21	401	16.71	463	19.29	238	9.92	393	16.38	285	11.88	**2,294**	**13.65**
An. moucheti	292	12.17	99	4.13	164	6.83	193	8.04	208	8.67	246	10.25	92	3.83	**1,294**	**7.70**
An. marshallii	71	2.96	39	1.63	9	0.38	0	0.00	0	0.00	0	0.00	0	0.00	**119**	**0.71**
An. nili	142	5.92	6	0.25	3	0.13	0	0.00	6	0.25	10	0.42	60	2.50	**227**	**1.35**

Table B5: Human Biting Rate of *Anopheles* Mosquitoes from Bonabéri using HLCs (October 2019-September 2020)

Anopheles species	Oct-19		Dec-19		Feb-20		Jun-20		Jul-20		Aug-20		Sep-20		Total	
	TC	HBR	TC	HBR	TC	HBR	TC	HBR	TC	HBR	TC	HBR	TC	HBR	TC	HBR
An. gambiae s.l.	249	10.38	40	1.67	46	1.92	658	27.42	837	34.88	183	7.63	113	4.71	**2,126**	**12.65**

TC=Total Collected, HBR=Human Biting Rate

Table B6: Human Biting Rate of *Anopheles* Mosquitoes and Endophagic Index in Simatou (October 2019-September 2020)

Anopheles species	Nov-19			Jan-20			Mar-20			Jun-20			Jul-20			Aug-20			Sep-20			Total		
	HBR in	HBR out	EI	HBR in	HBR out	EI	HBR in	HBR out	EI	HBR in	HBR out	EI	HBR in	HBR out	EI	HBR in	HBR out	EI	HBR in	HBR out	EI	HBR in	HBR out	EI
An. gambiae s.l.	3.50	5.00	0.41	2.50	1.17	0.68	87.92	84.25	0.51	25.67	15.25	0.63	352.33	302.00	0.54	192.92	207.25	0.48	75.00	66.08	0.53	105.69	97.29	0.52
An. ziemanni	0.00	0.08	0.00	0.00	0.00	0.00	0.50	0.17	0.75	0.33	0.17	0.67	0.75	0.08	0.90	4.25	3.25	0.57	16.92	15.58	0.52	3.25	2.76	0.54
An. funestus s.l.	1.58	2.17	0.42	0.00	0.17	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.08	0.00	1.00	0.24	0.33	0.41
An. pharoensis	0.58	0.25	0.70	1.75	2.00	0.47	38.92	37.83	0.51	4.83	4.50	0.52	0.75	2.08	0.26	24.33	18.67	0.57	9.83	8.67	0.53	11.57	10.57	0.52
An. rufipes	0.08	0.08	0.50	0.00	0.00	0.00	0.08	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.08	0.17	0.33	0.04	0.04	0.50
An. welcomei	0.00	0.08	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00
An. demeilloni	0.92	1.00	0.48	0.00	0.00	0.00	0.92	0.25	0.79	0.58	0.25	0.70	0.00	0.00	0.00	0.83	0.33	0.71	0.17	0.08	0.67	0.49	0.27	0.64

Table B7: Human Biting Rate of *Anopheles* Mosquitoes and Endophagic Index in Gounougou (October 2019-September 2020)

Anopheles species	Nov-19			Jan-20			Mar-20			Jun-20			Jul-20			Aug-20			Sep-20			Total		
	HBR in	HBR out	EI	HBR in	HBR out	EI	HBR in	HBR out	EI	HBR in	HBR out	EI	HBR in	HBR out	EI	HBR in	HBR out	EI	HBR in	HBR out	EI	HBR in	HBR out	EI
An. gambiae s.l.	21.42	17.08	0.56	14.67	15.17	0.49	36.92	39.75	0.48	23.92	21.92	0.52	63.58	60.00	0.51	45.50	50.67	0.47	59.33	64.75	0.48	37.90	38.48	0.5
An. ziemanni	0.17	0.33	0.33	0.08	0.08	0.50	0.00	0.17	0.00	0.17	0.42	0.29	0.33	0.42	0.44	0.17	0.42	0.29	0.25	0.75	0.25	0.17	0.37	0.31
An. funestus s.l.	10.67	4.83	0.69	1.75	0.67	0.72	0.00	0.00	0.00	0.50	0.50	0.50	0.00	0.25	0.00	0.17	0.00	1.00	1.08	1.58	0.41	2.02	1.12	0.64
An. pharoensis	0.00	0.00	0.00	0.17	0.50	0.25	2.75	5.00	0.35	0.00	0.00	0.00	0.08	0.08	0.50	0.00	0.00	0.00	0.08	1.50	0.05	0.44	1.01	0.3
An. rufipes	0.00	0.08	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.08	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0
An. multicinctus	0.17	0.08	0.67	1.00	1.25	0.44	0.33	0.33	0.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.21	0.24	0.47

HBR=Human Biting Rate, EI=Endophagic Index

Table B8: Human Biting Rate of *Anopheles* Mosquitoes and Endophagic Index in Mangoum (October 2019-September 2020)

Anopheles species	Oct-19			Dec-19			Feb-20			Jun-20			Jul-20			Aug-20			Sep-20			Total		
	HBR in	HBR out	EI	HBR in	HBR out	EI	HBR in	HBR out	EI	HBR in	HBR out	EI	HBR in	HBR out	EI	HBR in	HBR out	EI	HBR in	HBR out	EI	HBR in	HBR out	EI
An. gambiae s.l.	13.50	18.08	0.43	10.58	11.17	0.49	9.83	13.08	0.43	11.50	14.25	0.45	8.92	8.67	0.51	3.00	3.42	0.47	6.67	3.58	0.65	9.14	10.32	0.46
An. ziemanni	0.42	1.67	0.20	0.08	0.00	1.00	0.00	0.00	0.00	0.08	0.00	1.00	0.17	0.08	0.67	0.00	0.33	0.00	0.58	2.75	0.18	0.19	0.69	0.21

Table B9: Human Biting Rate of *Anopheles* Mosquitoes and Endophagic Index in Nyabessang (October 2019-September 2020)

Anopheles species	Oct-19			Dec-19			Feb-20			Jun-20			Jul-20			Aug-20			Sep-20			Total		
	HBR in	HBR out	EI	HBR in	HBR out	EI	HBR in	HBR out	EI	HBR in	HBR out	EI	HBR in	HBR out	EI	HBR in	HBR out	EI	HBR in	HBR out	EI	HBR in	HBR out	EI
An. gambiae s.l.	2.25	4.83	0.32	1.00	1.08	0.48	0.08	0.00	1.00	2.67	0.92	0.74	0.00	0.00	0.00	0.17	0.17	0.50	0.83	2.00	0.29	1.00	1.29	0.43
An. paludis	5.83	6.58	0.47	10.50	19.92	0.35	15.00	18.42	0.45	19.67	18.92	0.51	11.00	8.83	0.55	15.83	16.92	0.48	10.75	13.00	0.45	12.65	14.65	0.46
An. moucheti	14.83	9.50	0.61	4.58	3.67	0.56	8.50	5.17	0.62	9.25	6.83	0.58	7.50	9.83	0.43	9.83	10.67	0.48	3.92	3.75	0.51	8.35	7.06	0.54
An. marshallii	3.50	2.42	0.59	2.67	0.58	0.82	0.42	0.33	0.56	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.94	0.48	0.66
An. nili	4.83	7.00	0.41	0.33	0.17	0.67	0.17	0.08	0.67	0.00	0.00	0.00	0.33	0.17	0.67	0.25	0.58	0.30	2.67	2.33	0.53	1.23	1.48	0.45

Table B10: Human Biting Rate of *Anopheles* Mosquitoes and Endophagic Index in Bonabéri (October 2019-September 2020)

Anopheles species	Oct-19			Dec-19			Feb-20			Jun-20			Jul-20			Aug-20			Sep-20			Total		
	HBR in	HBR out	EI	HBR in	HBR out	EI	HBR in	HBR out	EI	HBR in	HBR out	EI	HBR in	HBR out	EI	HBR in	HBR out	EI	HBR in	HBR out	EI	HBR in	HBR out	EI
An. gambiae s.l.	7.25	13.50	0.35	1.33	2.00	0.40	0.83	3.00	0.22	21.42	33.42	0.39	23.08	46.67	0.33	5.42	9.83	0.36	3.08	6.33	0.33	8.92	16.39	0.35

HBR=Human Biting Rate, EI=Endophagic Index

ANNEX C: HUMAN BLOOD INDEX, PARITY, AND INFECTION RATES BY SITE

Table C1: Human Blood Index of *Anopheles* Mosquitoes across Sentinel Sites

Sites	Host	*An. gambiae* s.l.	*An. funestus* s.l.	*An. rufipes*	*An. demeilloni*	*An. multi-cinctus*	*An. pharoensis*	*An. ziemanni*	Total	HBI
Gounougou	Human	356	10	0	0	0	0	0	366	50.41%
	Animal	239	4	0	0	0	0	0	243	
	Mix	21	0	0	0	0	0	0	21	
	Not identified	96	0	0	0	0	0	0	96	
	Total	712	14	0	0	0	0	0	726	
Simatou	Human	252	3	1	23	0	0	0	279	46.89%
	Animal	69	2	9	1	0	0	0	81	
	Mix	72	0	3	4	0	0	0	79	
	Not identified	146	0	9	1	0	0	0	156	
	Total	539	5	22	29	0	0	0	595	
Mangoum	Human	63	0	0	0	0	0	0	63	94.03%
	Animal	0	0	0	0	0	0	0	0	
	Mix	4	0	0	0	0	0	0	4	
	Total	67	0	0	0	0	0	0	67	
Nyabessang	Human	9	0	0	0	0	0	0	9	-
	Animal	0	0	0	0	0	0	0	0	
	Mix	0	0	0	0	0	0	0	0	
	Not identified	8	0	0	0	0	0	0	8	
	Total	17	0	0	0	0	0	0	17	
Bonabéri	Human	2	0	0	0	0	0	0	2	-
	Animal	0	0	0	0	0	0	0	0	
	Mix	0	0	0	0	0	0	0	0	
	Total	**2**	**0**	**0**	**0**	**0**	**0**	**0**	2	

Table C2: Number of Ovaries Dissected and Parity Rate by Species and Site

Anopheles species	Ovaries dissected	# Parous	Parity Rate
Simatou			
An. gambiae s.l.	2,123	1,621	76.35
An. ziemanni	1	1	100.00
An. funestus s.l.	38	36	94.74
An. pharoensis	76	58	76.32
An. rufipes	2	2	100.00
An. welcomei	0	0	0.00
An. demeilloni	41	35	85.37
Total Simatou	**2,281**	**1,753**	**76.85**
Gounougou			
An. gambiae s.l.	1,104	778	70.47
An. ziemanni	7	5	71.43
An. funestus s.l.	88	70	79.55
An. pharoensis	20	14	70.00
An. rufipes	0	0	0.00
An. multicinctus	21	18	85.71
Total Gounougou	1,240	885	71.37
Nyabessang			
An. gambiae s.l.	74	48	64.86
An. paludis	305	187	61.31
An. moucheti	487	334	68.58
An. marshallii	63	34	53.97
An. nili	47	36	76.60
Total Nyabessang	**976**	**639**	**65.47**
Bonabéri			
An. gambiae s.l.	887	602	67.87
Total Bonabéri	**887**	**602**	**67.87**
Mangoum			
An. gambiae s.l.	362	268	74.03
An. ziemanni	14	6	42.86
An. funestus s.l.	0	0	0.00
Total Mangoum	**376**	**274**	**72.87**
Total	**5,760**	**4,153**	**72.10**

Table C3: Infection Rate of *Anopheles* Mosquitoes by Site (October 2019-July 2020)

Species	Simatou			Gounougou			Bonabéri			Mangoum			Nyabessang			Total		
	# Tested	# Positive	% Infection	# Tested	# Positive	% Infection	# Tested	# Positive	% Infection	# Tested	# Positive	% Infection	# Tested	# Positive	% Infection	# Tested	# Positive	% Infection
An. gambiae s.l.	1,373	6	0.43	1381	17	1.23	866	14	1.61	993	28	2.81	133	10	7.51	4,746	75	1.58
An. funestus s.l.	28	2	7.14	62	1	1.61										90	3	3.33
An. pharoensis	388	3	0.7	17	0	0.00										405	3	0.74
An. ziemanni	9	0	0	27	2	7.40				59	2	3.38				95	4	4.21
An. multicinctus				59	2	3.39										59	2	3.39
An. moucheti													565	5	0.88	565	5	0.88
An. nili													79	3	3.79	79	3	3.79
An. marshallii													65	1	1.53	65	1	1.53
An. paludis													656	9	1.37	656	9	1.37
An. rufipes	2	1	50	2	0	0										4	1	-
TOTAL	1,800	12	0.66	1,548	22	1.42	866	14	1.61	1052	30	2.85	1,498	28	1,86	6,764	106	1.56

ANNEX D: WHO SUSCEPTIBILITY TEST AND CDC BOTTLE RESULTS

Table D1: WHO Susceptibility Test Results across Sites in 2020

Insecticide	Simatou		Gounougou		Mangoum		Nyabessang		Bonabéri	
	Total exposed	% Mortality	Total exposed	% Mortality	Total exposed	% Mortality	Total exposed	% Mortality	Total exposed	% Mortality
Pirimiphos-methyl 1x	97	100	93	100	83	96.4	92	100	100	100
Permethrin 1x	86	28.2	105	6.59	83	0	92	10.8	100	1
Permethrin 5x	87	65.7	91	55.2	85	30.6	87	65.7	100	90
Permethrin 10x	94	71.1	92	90.7	83	82	81	87.6	100	100
PBO + Permethrin	90	79.6	94	24.8	79	2.5	90	35.5	94	37
Deltamethrin 1x	86	46.6	99	20.6	80	28.8	89	23.7	96	76
Deltamethrin 5x	87	65.7	107	28.9	82	35.7	98	23.7	92	89
Deltamethrin 10x	94	71.1	85	66.2	82	47.4	84	91.7	100	100
PBO + Deltamethrin	90	44.3	105	88.5	83	51.2	96	84.1	100	95
Alpha-cypermethrin 1x	81	28.3	102	9.7	82	3.6	90	14.4	100	52
Alpha-cypermethrin 5x	92	33.4	105	23.7	84	5.8	92	28	100	77
Alpha-cypermethrin 10x	88	53.9	90	32.8	84	27.5	95	50.4	100	99
PBO + Alpha-cypermethrin	86	62.8	100	38.3	82	45.2	88	88.7	100	100
Bendiocarb 1x	92	100	93	100	84	70.3	99	88.9	100	100

Table D2: WHO Susceptibility Test Results with Clothianidin across Sites in 2020

Times (hours)	Simatou		Gounougou		Mangoum		Nyabessang		Bonabéri	
	Total exposed	% mortality	Total exposed	% mortality	Total exposed	% mortality	Total exposed	% mortality	Total exposed	% mortality
J1 (24 hours)	86	95.4	98	98.9	82	75.5	95	94.6	100	97
J2	86	95.4	98	98.9	82	81.6	95	100	100	100
J3	86	100	98	100	82	84	-	-	-	-
J4	-	-	-	-	82	90.1	-	-	-	-
J5	-	-	-	-	82	93.8	-	-	-	-
J6	-	-	-	-	82	96.3	-	-	-	-
J7	-	-	-	-	82	98.8	-	-	-	-

Table D3: CDC Bottle Susceptibility Test Results with Chlorfenapyr 100 µg across Sites in 2020

Times (hours)	Simatou		Gounougou		Mangoum		Nyabessang		Bonabéri	
	Total exposed	% mortality	Total exposed	% mortality	Total exposed	% mortality	Total exposed	% mortality	Total exposed	% mortality
J1(24 hours)	100	87	87	92.2	98	100	97	95.9	100	84
J2	100	97	87	98.8	98	100	97	100	100	99
J3	100	100	87	98.8	98	100	97	100	100	99

Table D4: Frequency of Target Site Resistance Allele across Sites

Site	N1575Y			*Kdr*-w			*Kdr*-e			*Ace*-1		
	RR	RS	SS	RR	RS	SS	RR	RS	SS	RR	RS	SS
Gounougou	1	28	67	36	44	20	0	71	29	0	0	102
Simatou	1	63	36	11	73	16	1	12	83	0	0	92
Mangoum	3	20	51	98	2	0	0	1	96	44	0	53
Nyabessang	0	6	94	25	75	0	1	20	79	6	1	92
Bonabéri	0	5	95	93	6	1	0	6	94	1	3	95
Total	5	122	343	263	200	37	2	110	381	51	4	434

ANNEX E: BIBLIOGRAPHY

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