THE PMI VECTORLINK SENEGAL PROJECT

ANNUAL ENTOMOLOGICAL REPORT

JANUARY 2021-DECEMBER 2021

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Acronyms

b/p/n	bite/person/night
CDC	Centers for Disease Control and Prevention
EIR	Entomological Inoculation Rate
ELISA	Enzyme-Linked Immunosorbent Assay
f/r	female per room
HBR	Human Biting Rate
HLC	Human Landing Catch
IRD	Indoor Resting Density
IRS	Indoor Residual Spraying
ITN	insecticide-treated net
LEVP	Laboratoire d'Ecologie Vectorielle et Parasitaire
NMCP	National Malaria Control Programme
PMI	President's Malaria Initiative
UCAD	Université Cheick Anta Diop
WHO	World Health Organization

EXECUTIVE SUMMARY

In Senegal, the main malaria vector control interventions include implementing indoor residual spraying (IRS) and distribution of insecticide-treated nets (ITNs). The U.S. President's Malaria Initiative (PMI) VectorLink Project, funded by the U.S. Agency for International Development (USAID), supports the implementation of both interventions in Senegal.

In 2021, VectorLink Senegal supported the National Malaria Control Programme (NMCP) to implement Indoor Residual Spraying (IRS) in four districts including Kédougou, Makacolibantang, Koumpentoum, and Koungheul. A single spray campaign was conducted in all districts from May 31 through June 29, 2021, using two clothianidin-based formulations (SumiShield in Makacolibantang, Koumpentoum and Koungheul and Fludora Fusion in Kedougou, and partly in Koumpentoum and Koungheul). During IRS campaign, spray operators enumerated a total of 145,870 structures of which they sprayed 141,717, for a spray coverage rate of 97.2%) PMI VectorLink, in collaboration with the NMCP and PMI, distributed 1,148,292 pyrethroid-only ITNs (22,966 bales) in 14 regions in 2021, and in 2020 distributed 10,015 piperonyl butoxide ITNs across seven health posts in Tambacounda district, as part of a Mass Drug Administration operational research study. In 2021, PMI VectorLink Senegal also supported the routine distribution of pyrethroid-only ITNs in 79 districts across the 14 regions and 79 districts of the country. Overall, a total of 1,148,292, ITNs were distributed to households from January to December 2021 through health facilities and community-based organizations**.**

In order to assess the effectiveness and impact of these vector control interventions, PMI VectorLink Senegal and its subcontractor, the Laboratoire d'Ecologie Vectorielle et Parasitaire (LEVP) of Cheikh Anta Diop University (UCAD) conducted entomological monitoring activities in selected sentinel sites across the country. Longitudinal vector surveillance and insecticide resistance monitoring was conducted in 34 sentinel sites spread across the five different geographical zones (Sahelian, Sahelo-Sudanese, Sudanese, Sudano-Sahelian and Sudano-Guinean zones), within the 19 health districts of the country (Figure 1) from January to December 2021, with the exception of March and April where collection wasn't done due to drier season. Adult mosquito collections were conducted monthly using human landing catches (HLCs) and pyrethrum spray catches (PSCs). Subsamples of preserved *An. gambiae* s.l. and *An. funestus* s.l. were screened for the presence of *Plasmodium falciparum* (*Pf*) infection and for species identification of each vector complex using enzyme-linked immunosorbent assay (ELISA) and polymerase chain reaction (PCR) respectively.

In the IRS sites, additional entomological activities included the monitoring of the quality of spray within a week after the campaign started followed by monthly assessment of the residual efficacy of the sprayed insecticides using World Health Organization (WHO) wall cone bioassays, until mortality of exposed mosquitoes drops below 80% for two successive months.

Insecticide resistance monitoring was also conducted once a year using females of *An. gambiae* s.l. reared from wild collected larvae per site. Insecticide susceptibility testing was conducted between October and November 2021 using the WHO tube test for adult mosquitoes and the U.S. Centers for Disease Control and Prevention (CDC) bottle test methods. Susceptibility status, resistance intensity and piperonyl butoxide (PBO) synergism of pyrethroid insecticides (alpha-cypermethrin 0.05%, deltamethrin 0.05% and permethrin 0.75%) and pirimiphos-methyl 0.25% and bendiocarb 0.1% was determined using WHO test kits. Chlorfenapyr 100 µg/bottle and clothianidin 4 µg/bottle were tested using CDC bottle assays.

Overall, a total of 14,023 *Anopheles* mosquitoes were collected from June to December 2021, including eight species (*An. gambiae* s.l., *An. funestus* s.l., *An. pharoensis*, *An. rufipes*, *An. squamosus*, *An. nili*, *An. coustani*, and *An. ziemanni*). *Anopheles gambiae* s.l. was the main vector collected at all zones (n = 11,069; 78.9%). Furthermore, *An. arabiensis* constituted the predominant and widespread species of the *An. gambiae* complex in four of the five geographical zones; except in the Sudano-Guinean zone dominated by *An. gambiae* s.s. The presence of *An. coluzzii* was also noted in all the surveyed areas, with the highest proportion in the Sudanese zone. Few hybrids of *An. gambiae/coluzzii* were recorded mainly in the Sudanese and Sudan-Guinean zones. Few specimens of *An. melas* were identified among the samples analyzed in the Sudan-Sahelian zone.

Anopheles funestus s.l. (n=2,506, 17.9%) represented the second mostly collected vector in the country and mainly in Ndoffane, located in the Sudano-Sahelian zone of the country. The subspecies of the complex included *An. funestus* s.s. and *An. rivulorum* recorded in three of the four sites tested.

In the IRS sites of the health districts of Kedougou, Makacolibantang, Koungheul and Koumpentoum, *An. gambiae* s.l. accounted for 98.0% (n = 3,832) of the total of the *Anopheles* mosquitoes collected from all IRS sites (n = 3,912) followed by *An. rufipes* (1.1%, n=43). The non-IRS control sites in Malem Hodar, Saraya, Salemata, and Tambacounda recorded a total of 4,094 *An. gambiae* s.l. (95.7%) over the total number of the *Anopheles* mosquitoes collected (n = 4,367).

The most diverse *Anopheles* species were recorded in Kédougou, with six species detected. Similar to the IRS districts, *An. gambiae* s.l. represented more than 94% of collections in control sites. *An. nili* was the second most common *Anopheles* species collected in the control sites of Kedougou with about 7.9% (122/1,543) of the *Anopheles* mosquitoes collected in Salemata. *An. arabiensis* was the main species (51.8%), of the *An. gambiae* complex in all IRS and control sites.

Overall, the mean human biting rate (HBR) of *An. gambiae* s.l. was less than 3.0 bites per person per night (b/p/n) across all geographical zones, with the highest in the Sudano-Guinean ((9.0 b/p/n) followed by the Sudano-Sahelian zones (3.7 b/p/n) while the lowest HBR was recorded in the Sahelo-Sudanese zone (0.3 b/p/n). The mean endophagic rate showed a slightly higher outdoor biting of *An. gambiae* s.l. at all geographical zones, except in the Sudano-Guinean (0.50%) and Sudanese zones (0.48%) where the vectors bite similarly indoors and outdoors. Also, the highest HBRs were recorded during the rainy season period (August to October) September within all geographical areas. The mean peak hourly biting occurred during the second half of the night both indoors and outdoors. Like the previous years, the mean HBR of *An. funestus* s.l. was very low (< 1.0 b/p/n) at all sites and geographical zones, except in Ndoffane (13.7 b/p/n) located in the Sahelo-Sahelian zone with a peak of 22.7 b/p/n in July 2021 recorded outdoors. *An. funestus* s.l. remains exophagic (0.59) during the collection period.

At all IRS sites, *An. gambiae* s.l. bites more outdoors than indoors while in control sites indoor and outdoor biting rates were similar. The average indoor and outdoor HBR in Kedougou sprayed with Fludora Fusion, was higher (4.9 b/p/n) than that recorded in the three other sites sprayed with SumiShield (0.9 b/p/n). The mean indoor/outdoor HBR in the unsprayed sites was higher (>14 b/p/n) in three of the sites (Tambacounda, Saraya and Salémata) except in Malem Hodar recording less than 1.5 b/p/n both indoors and outdoors. The peak biting was also observed between August and October 2021 at all IRS and control sites.

The mean indoor resting density (IRD) of *An. gambiae* s.l. expressed as the number of females per room (f/r) was approximately of 1.4 f/r for the entire monitoring period. The highest IRDs were recorded in Sudan-Sahelian (2.7 f/r) zones, while the lowest IRD was recorded in the Sudan-Guinean zone (0.6 f/p).

EIR of *An. gambiae* s.l. populations varied according to the geographical zones and was higher in the Sudanese and the Sudano-Guinean zones. Furthermore, higher EIR was recorded in each control site compared to its sprayed site, except in Koungheul where no infected mosquito was recorded in both IRS and control sites. However, the EIRs were still low in all sites (between 0.005 infected b/p/n in Koumpentoum and 0.122 ib/p/n in Kedougou for IRS sites, and between 0.021 in Koussanar (control Koumpentoum) and 0.208 ib/p/n in Sareya and Salemata (control Kedougou). Infected females were found in *An. arabiensis*, *An. gambiae* s.s. and *An. coluzzii*, and the highest levels of infection recorded were obtained in *An. gambiae* s.s. in the Sudano-Guinean zone One female out of the 18 of *An. funestus* s.l. tested was infected in Salemata.

The spray quality of both Fludora Fusion and Sumishield in the sprayed sites was good. The results indicate that both insecticides showed a high residual efficacy with an average mortality rate above 99%, in all treated wall types (mud and cement) and sites during eight months.

Anopheles gambiae s.l. was resistant to all pyrethroids in the sites where the tests were conducted with varying levels of intensity of e resistance. Low resistance intensity to deltamethrin and permethrin was detected in Kedougou and moderate resistance to deltamethrin, alpha-cypermethrin and permethrin was detected in all the other sites. The PBO synergist assay test was conducted in nine sentinel districts including IRS and PBO-ITN distribution sites. Pre-exposure to PBO substantially increase mortality of all pyrethroids with reversal to full susceptibility of the *An. gambiae* s.l. population of Koungheul, Koumpentoum and Tambacounda to pyrethroids. Susceptibility to pirimiphos-methyl was recorded in all sites but for bendiocarb, only *An. gambiae* s.l. population of Koungheul was susceptible. Susceptibility of *An. gambiae* s.l. to clothianidin 4 µg/bottle was recorded at all sites tested, except at Koumpentoum (95.5%). Susceptibility of *An. gambiae* s. l. was recorded against chlorfenapyr 100 µg/bottle at 11 out of 12 sites surveyed.

The entomological monitoring data collected annually provides the Senegal NMCP with updated data to guide the selection of appropriate vector control tool deployment.

1. INTRODUCTION

In Senegal, malaria remains endemic and represents a major cause of morbidity and mortality particularly among children under 5 years and pregnant women and represents a high priority for the government. During the past two decades, the government of Senegal, supported by its partners and key stakeholders, has put substantial efforts on vector control and malaria treatment to reduce the burden within the populations at risk. As part of an effort to scale up vector control interventions, the Senegal NMCP has received support from PMI for IRS, ITN distributions, and entomological monitoring since 2007. In 2020, after a two-year pause, Senegal resumed IRS with the introduction of two new insecticides (Fludora Fusion in Koungheul, Koumpentoum, and Makacolbanta, and SumiShield in Kédougou). In 2021, IRS was conducted in the same districts and insecticides, but which were rotated (Fludora Fusion in Kédougou and SumiShield in Koungheul, Koumpentoum, and Makacolbanta). Furthermore, PMI VectorLink, in collaboration with the NMCP and PMI, distributed 1,148,292 standards ITNs (pyrethroid-only) (22,966 bales) in 14 regions, and in 2020 distributed 10,015 piperonyl butoxide ITNs across seven health posts in Tambacounda district, as part of a Mass Drug Administration operational research study.

In 2021, PMI VectorLink Senegal conducted monthly longitudinal entomological monitoring activities in collaboration with the Laboratory of Vector and Parasite Ecology (Laboratoire d'Ecologie Vectorielle et Parasitaire) of the Faculty of Sciences and Techniques (Faculté des Sciences et Techniques) of Cheikh Anta Diop University (Université Cheikh Anta Diop, UCAD) in Dakar. These activities included IRS monitoring in eight sites, vector surveillance in 24 sentinel districts spread across the different eco-geographical zones of the country, and annual insecticide resistance monitoring in 12 districts. Additionally, cone bioassays were conducted to assess the quality of the spray during the IRS campaign and monthly insecticide decay monitoring.

The data collected will be used for decision-making by the NMCP and the malaria vector control stakeholders (including PMI VectorLink) about the timing of IRS campaigns and in the selection and distribution of ITNs.

2. METHODS

2.1 ENTOMOLOGICAL MONITORING SITES

In 2021, PMI VectorLink Senegal conducted monthly longitudinal entomological monitoring activities in collaboration with the Laboratory of Vector and Parasite Ecology (Laboratoire d'Ecologie Vectorielle et Parasitaire) of the Faculty of Sciences and Techniques (Faculté des Sciences et Techniques) of Cheikh Anta Diop University (Université Cheikh Anta Diop, UCAD) in Dakar. In January and February 2021 and from May to December 2021, VectorLink Senegal conducted entomological monitoring in 34 sites across 19 districts of the country (Figure 1) including longitudinal vector surveillance and insecticide resistance monitoring. Sentinel sites were selected within the districts across the different geographical zones of the country. Data collection started in January in some sites and stopped between March and April due to the dry season. Tivaouane (2 sites) and Oussouye (2 sites) were sites where entomological activities were conducted in 2020 (Year 3 workplan sites) and were monitored through February 2021. They were later excluded from the May-September 2021 activities, for a remaining 30 sites monitored from May to December 2021. (Table 1).

Longitudinal vector surveillance in 2021 was conducted in 30 sites including eight indoor residual spraying (IRS) sites, six associated control sites and four PBO net distribution sites. The PBO net sites included Kouthia Farinala Manding (health post Koussanar), Velingara Sabakel (heath post Sinthiou Malem), Oundoundou (heath post Dar Salam) and Safalou 1 (heath post Missirah) selected in Tambacounda district where PBO insecticide-treated nets (ITN) were distributed. Pyrethroid-only ITNs were distributed in the other 14 routine entomological data collection sites. (Table 1).

A specific study was done in Dakar center to look for *An. stephensi* presence.

Figure 1: Sentinel Districts for Entomological Surveillance Activities in The Different Geographical Zones

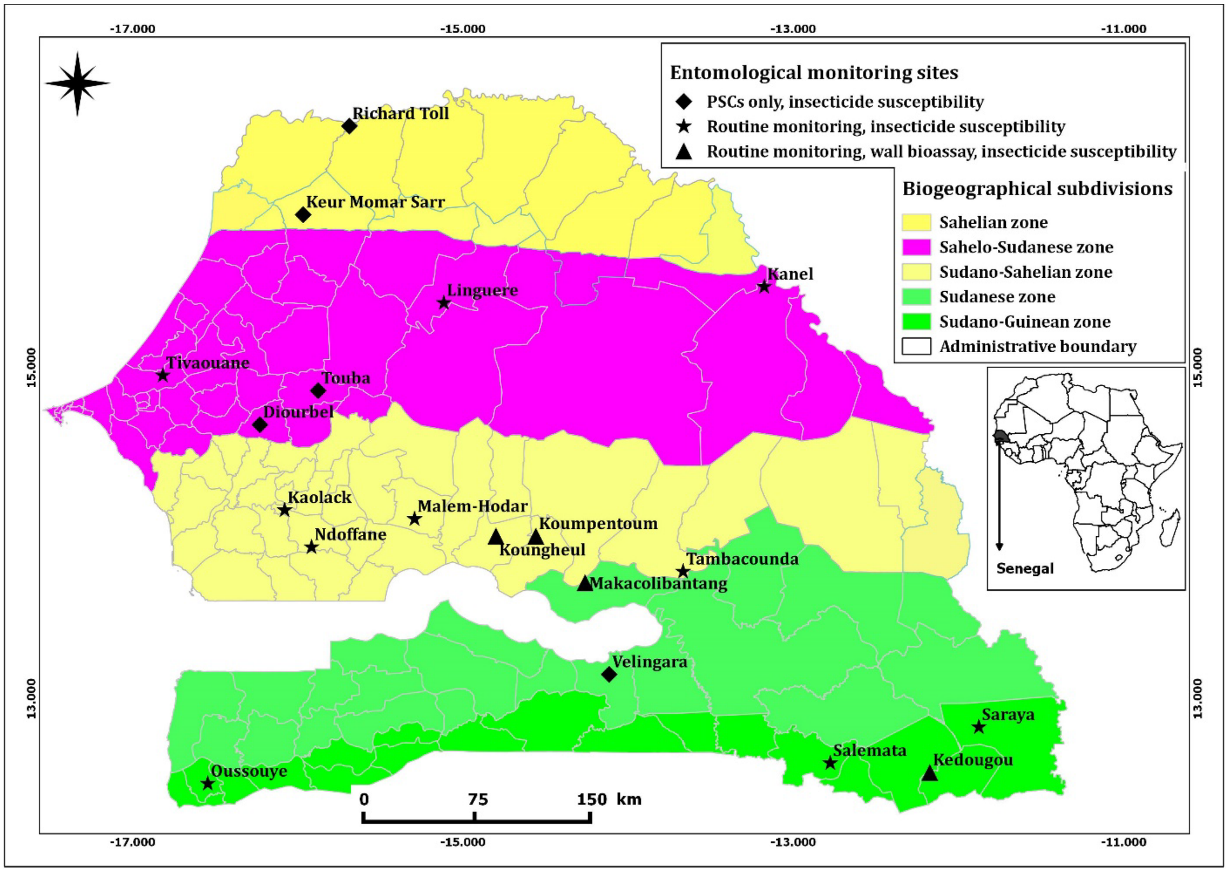


Table 1: Sentinel Districts, Entomological Activities Per Site and Collection Timeline

Sentinel Districts	Sentinel Sites	Monitoring Activities	Status
January to September 2021			
Sahelian area			
Richard-Toll‡	1) Mbagame	**PSCs, IS****	Conducted every month and started in May 2021
Keur Momar Sarr	2) Gankette Balla		
Sahelo-soudanese area			
Kanel	3) Haouré, 4) Dembankané	**HLCs, PSCs, IS****	Conducted every month and started in May 2021
Linguere	5) Barkedji, 6) Ouarkhokh	**HLCs, PSCs, IS****	
Tivaouane	7) Keur Mbirndao, 8) Sawo Mekhe	**PSCs, IS****	February 2021
Sudano-Sahelian area			
Touba‡	9) Héliport	**PSCs, IS****	Conducted every month and started in June 2021
Diourbel‡	10) Keur SérigneMbaye Sarr	**PSCs, IS****	
Koungheul≠	11) Ida Mouride, 12) Pakala	**WB, HLCs, PSCs, IS****	

Malem Hodar*	13) Maka Belal, 14) Ndiote Mor Coumba	**HLCs, PSCs, IS****	Conducted in January and February and monthly from May 2021
Kaolack	15) Ndorong	**HLCs, PSCs, IS****	Conducted in February and monthly from June 2021
Ndoffane	16) Tawa Mboudaye	**HLCs, PSCs, IS****	
	Sudanese area		
Koumpentoumǂ	17) Darou Salam 2, 18) Kouthiaba	**WB, HLCs, PSCs, IS****	Conducted in January and February 2021 and monthly from June 2021
Makacolibantangǂ	19) Sinthiou Bouré Banna Ndao, 20) Souarécounda	**WB, HLCs, PSCs, IS****	
Tambacounda*	21) Koussanar, 22) Lycounda, 23) Vélingara Sabaké, 24) Kouthia Farindella, 25) Oundoundou, 26) Safalou 1	**HLCs, PSCs, IS****	
Sudano-Guinean area			
Kédougouǂ	27) Tomboronkoto, 28) Bandafassi	**WB, HLCs, PSCs, IS****	Conducted in January and February 2021 and monthly from June 2021
Saraya	29) Bembou	**HLCs, PSCs, IS****	
Salemata	30) Diara Pont	**HLCs, PSCs, IS****	
Vélingara‡	31) Medina Dianguette, 32) Bonkonto	**PSCs, IS****	Conducted in February 2021 and monthly from June 2021
Oussouye	33) Cadjinolle, 34) Mlomp	**PSCs, IS****	January – February 2021

Note: PSCs = pyrethroid spray catches, WB = wall bioassay, HLCs = human landing catches, IS = insecticide susceptibility
ǂ = PMI IRS-sites, * = unsprayed control, **IS only done during peak rainy season
‡ = planned community-based surveillance and not conducted due to COVID-19

2.2 Longitudinal Monitoring of Malaria Vectors Density and Behavior

Adult mosquitoes were collected every month from May to December 2021, through routine monitoring. The monitoring used PSCs for indoor resting females (endophilic) in human habitations (Standard Operating Procedure 03/01), and hourly HLCs of host-seeking mosquito females inside and outside human dwellings (Standard Operating Procedure 02/01) and and outdoor resting collection (ORC) using prokopack aspirators. Collections were in 30 villages (8 sprayed villages, 18 unsprayed villages and 4 piperonyl butoxide-ITN villages) in the district of Tambacounda. Six of these villages were used as unsprayed controls, and four were IRS sites. All entomological data collections were conducted following PMI standard operation procedures that can be found here https://pmivectorlink.org/resources/tools-and-innovations/.

Sampling methods and entomological indicators per collection method are presented in Tables 2 and 3, respectively. The same rooms and houses were maintained over the survey period.

Table 2: Longitudinal monitoring adult mosquito collection methods

Collection method	Time	Frequency	Sample
PSC	7:00 am to 9:00 am	One day per site per month	Ten (10) houses per site per month
HLC	8:00 pm to 6:00 am	Two successive nights per site per month	Three (3) houses per site (Indoor and outdoor)
ORC	7:00 am to 9:00 am	One day per site per month	Ten (10) artificial shelters

2.2.1 PYRETHRUM SPRAY CATCH

In each site where PSCs were conducted (17 districts), 10 houses were selected. PSCs were conducted from 7 a.m.to 9 a.m, for one day and once per month from May to December 2021. The same houses were visited each month. A commercial aerosol made of the pyrethroids d-tetramethrin 0.135% w/w, d-allethrin 0.06% w/w, and cypermethrin 0.46% w/w was used to knock down the mosquitoes. The room was closed for 10 minutes after spraying with an aerosol, and then the knocked-down mosquitoes were collected using forceps into a labeled petri dish. The samples were identified morphologically, sorted by abdominal status (blood-fed, gravid, or unfed), and preserved in 1.5 ml Eppendorf tubes with silica gel and kept in boxes at the laboratory for further species identification using the polymerase chain reaction technique.

2.2.2 HUMAN LANDING CATCH

HLCs were conducted in 24 sites. Three houses were sampled in each selected village during two consecutive nights to obtain 12 person-nights of collection per district per month (3 houses x 2 collection nights = 6 person-nights indoors and six person-nights outdoors). In all districts, two human volunteers (trained adult mosquito collectors) were positioned, one inside the house and the other outside at least 5 meters from the house, to collect mosquitoes. Collections were conducted from 8 p.m. to 6 a.m. using 12 volunteers working in shifts of five hours each to collect mosquitoes using hemolysis tubes. Collected mosquitoes were transferred into labeled bags assigned for each hourly collection. Collected mosquitoes were subsequently identified morphologically using the identification keys (Coetzee 2020). The mosquitoes collected were recorded by species, location, and hour of collection. All or a subsample of mosquitoes collected were dissected for parity. Either the mosquitoes or the carcasses of dissected mosquitoes were later preserved in 1.5 ml Eppendorf tubes with silica gel and kept in boxes for subsequent molecular analysis.

2.2.3 OUTDOOR RESTING COLLECTION

Outdoor resting collections (ORC) were performed using the prokopack aspirators in selected sites of Tambacounda district to collect exophilic mosquitoes. Collections were done in vegetation, open verandas, tree holes, open animal enclosures and eaves (Figure 2). Potential resting places were investigated and surveyed during one morning per collection period. The mosquitoes collected were morphologically identified and sorted by abdominal status. All vectors were preserved for further laboratory analysis.

Figure 2: Outdoor collection sites (A= shelter, B= tree hole, C= eave)

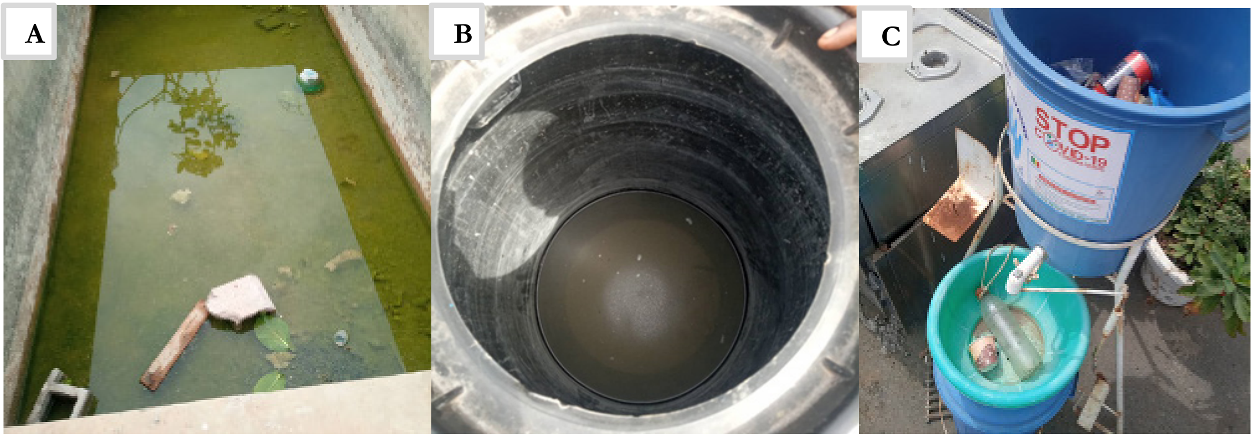
Table 3: Entomological indicators per collection method

Collection method	Indicator	Definition
HLC	Human biting rate (HBR) Indoor/Outdoor	Number of bites/person-night Indoor/Outdoor
	Peak biting time (PBT)	Hour with the highest human biting rate
	Parity rate (PR)	Percentage of parous mosquitoes
	Exophagic rate	Percentage of mosquitoes biting outside
	Endophagic rate	Percentage of mosquitoes biting inside
PSC	Indoor resting density (IRD)	Mean number of mosquitoes / house / days
	% fed females	Number of fed mosquitoes / totals collected
ORC	Outdoor resting density (ORD)	Mean number of mosquitoes /per shelter / days
	% fed females	Number of fed mosquitoes / totals collected

2.2.4 *ANOPHELES STEPHENSI* SURVEY IN DAKAR

Larval collections were conducted in breeding habitats areas in Dakar in October 2021 to assess the potential presence of *An. stephensi.* The survey was conducted around the the Autonomous Port of Dakar, Leopold Sedar Senghor airport and their surroundings. These sites were selected following *An. stephensi* invasion characteristics per previous reports (Sinka *et al*, 2020; Meshesha *et al*, 2020) The dipping method was used to collect *Anopheles* larvae found in reservoirs (Figure 3), sent to the insectary and reared to adults for morphological identification and molecular species identification for any suspected *An. stephensi.*

Figure 3: Type of breeding sites visited in the urban area of Dakar (A= opened gutter, B= opened water basin, C= abandoned bucket)



2.3 Laboratory Analysis

Morphologically identified *Anopheles gambiae* s.l. and *An. funestus* s.l. (Coetzee, 2020) mosquitoes collected during HLCs and PSCs were preserved on silica gel prior to laboratory analyses. Only samples collected from May to December were processed through enzyme-linked immunosorbent assay for circumsporozoite proteins (ELISA-CSP).

2.3.1 Molecular Identification of *An. gambiae* Species and Molecular Characterization of Target Site Resistance Genes

Sibling species of a subsample of *An. gambiae* complex (4,948) and *An. funestus* group (639) collected both by HLCs and PSCs were identified using the polymerase chain reaction technique as described by Wilkins et al. (2006). Additionally, the presence of the knock down resistance west and east allele mutations (*Kdr* (L1014F and L1014S)) and the acetylcholinesterase (*Ace-1* (G119S)) were screened among dead and alive specimens exposed to insecticides as respectively described by Huynh (2007) and Wilkins (2006).

2.3.2 Plasmodium Falciparum Infection Rate

The presence of *Plasmodium falciparum* circumsporozoite protein was characterized using the ELISA method (Burkot et al. 1984, Wirtz et al. 1987) to determine the infection rates among subsamples of vectors collected using both HLC and PSC methods. The circumsporozoite index was calculated as the proportion of females found with the *P. falciparum* circumsporozoite protein out of the total analyzed. The entomological inoculation rate (EIR) was calculated by multiplying the HBR by the circumsporozoite index.

2.4 Wall Bioassays

Four districts were sprayed during the 2021 Senegal IRS campaign conducted from May 31 to June 29, 2021. Kedougou was sprayed with Fludora Fusion Wettable Powder containing 500 g/kg clothianidin+62.5 g/kg deltamethrin while Makacolibantang, Koumpentoum and Koungheul were sprayed with SumiShield 50WG containing 50% w/w Water Dispersible Granule (300 mg ai/m^2).

Residual efficacy of insecticide-treated walls was assessed monthly using cone bioassays following the PMI VectorLink Standard Operating Procedure 09/01. Six houses in each of the two sprayed villages were randomly selected in each IRS-district. Five of them were sprayed and one unsprayed house served as the control. The houses were made of either mud or cement. Three cones were installed on three walls in each of the sprayed houses at 0.5 m, 1m, and 1.5m above the floor, and three cones at the control house. About 10 females, 2 to 5 days old, from the laboratory susceptible strain of *An. coluzzii* maintained in the insectary of LEVP, were exposed in each cone for 30 minutes and then transferred to holding cups for delayed mortality, recording up to five days post exposure. The residual efficacy life was monitored monthly until the mortality of the mosquitoes tested dropped below 80% for two consecutive months for all walls tested.

2.5 WHO Susceptibility Test

Susceptibility of adult *An. gambiae* s.l., the major malaria vector in Senegal, was assessed against different insecticides using the standard WHO susceptibility test kits, and CDC bottle assay procedures. Unfed adult females aged three to five days, reared from larvae collected from breeding sites within and around the sentinel sites, were used for the bioassays performed in the surveyed health districts. Diagnostic concentration of papers impregnated with four pyrethroids (deltamethrin 0.05%, permethrin 0.75%, alpha-cypermethrin 0.05% and lambda-cyhalothrin 0.05%), a carbamate (bendiocarb 0.1%), and an organophosphate (pirimiphos-methyl 0.25) were used to assess the susceptibility status of *An. gambiae* s.l. populations at resistance monitoring sites.

Insecticide susceptibility tests were completed following the WHO method (VectorLink Standard Operating Procedure 06/01), except for the tests with chlorfenapyr 100 µg/bottle and clothianidin 4 µg/bottle, which were performed using CDC bottle assays (VectorLink Standard Operating Procedure 04/01). The susceptibility testing was conducted as described above and the mortality was recorded up to 3 days post exposure for chlorfenapyr and 24h for clothianidin. When insecticide resistance of pyrethroids was confirmed, resistance intensity (high, moderate, and low) was also tested at 5x and 10x the diagnostic concentration of permethrin, deltamethrin, and alpha-cypermethrin, using the above WHO method.

Synergist assays with piperonyl butoxide (piperonyl butoxide 4%) were conducted for deltamethrin, permethrin, and alpha-cypermethrin according to the WHO tube test protocol (VectorLink Standard Operating Procedure 06/01) to determine the involvement of P450s in pyrethroid resistance.

Abbott's formula was used to correct the observed mortality in the cases where the control mortality was above 5% and below 20%. The results were interpreted based on the WHO criteria (2016).

2.6 Data Presentation and Interpretation

The District Health Information Software Version 2-based VectorLink Collect database was used for entomological data management in Senegal for the first time in 2020. The VectorLink home office staff remotely trained and supported UCAD and the project's entomologists and database managers on updated data workflows—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 Senegal in 2020 was analyzed using VectorLink Collect. The platform includes comprehensive dashboards to synthesize vector bionomics and insecticide resistance summary results.

Table 4: Interpretation of Insecticide Susceptibility Data

Status	WHO Threshold	Additional Thresholds	Resistance status
Susceptible	98–100%	98–100%	Susceptibility confirmed
Resistant	< 98%	90–97%	Resistance suspected
		< 90%	Resistance confirmed

When resistance to the diagnostic concentration of pyrethroids was observed, intensity of resistance was identified using the WHO susceptibility test (5x and 10x). The results were also interpreted in accordance with the WHO criteria of low, moderate, and high resistance intensity (WHO, 2016).

Table 5: Interpretation of Resistance Intensity Data

Resistance Intensity	Mortality at 5x	Mortality at 10x
Low resistance	98–100%	--
Moderate resistance	<98%	98–100%
High resistance	--	<98%

Homogeneity tests were performed to compare all the entomological parameters estimated for the two main vector species across their range of distribution, using the standard Chi-square or the exact Fisher tests where appropriate at the significance level of 0.05. The 95% confidence intervals were calculated for each *P. falciparum* infection rate.

3. RESULTS

3.1 VECTOR POPULATION DYNAMICS

3.1.1 GEOGRAPHICAL ZONE

3.1.1.1 SPECIES COMPOSITION

A total of 14,023 *Anopheles* mosquitoes, including eight different species (*An. gambiae* s.l., *An. funestus* s.l., *An. pharoensis*, *An. rufipes*, *An. nili*, *An. coustani, An. ziemanni and An. squamosus*), were collected in all sentinel districts of all geographical zones. *Anopheles gambiae* s.l. represented the main *Anopheles* and vector species collected (78.9% n=11,069), within the country, followed by *An. funestus* s.l (17.9%, n=2,506) (Figure 4).

The Sahelian and Sahelo-Sudanese zones recorded the lowest densities, with respectively 3.5% (n=487) and 3.7% (n= 512) of the total *Anopheles* collected, followed by the Sudanese zone (15.4%, n=2,153), the Sudano-Guinean zone (34.1%, n=4,779) and the Sudano-Sahelian zone (43.4%, n=6,092). As the number of sentinel sites are different from one zone to another, the density and percentage of mosquitoes collected can be correlated with the number of sentinel sites selected within each geographical zone. Most *Anopheles* species were found in the Sudano-Guinean zone (seven species), with *An. squamosus, An. coustani*, and *An. nili* found only in this zone. *Anopheles Gambiae* s.l., *An. funestus*, *An. pharoensis*, and *An. rufipes* were present in all the surveyed geographical zones. *An. ziemanni* was collected only in the Sahelo-Sudanese and Sahelian zone, at a very low percentage (0.04%; n=6). Except the Sahelian zone where *An. funetus* s.l. represented 82.8% (n=403) of the collected *Anopheles* mosquitoes, *An. gambiae* s.l. remained the predominant vector species in all other areas with density varying between 65.0% (n= 3,960) of *Anopheles* collected in the Sudano-Sahelian zone to 95.8% of the mosquitoes (n = 2,063) of the Sudanese zone (Figure 5). *An. funestus* s.l. constituted the second most prevalent species in the Sudano-Sahelian zone (33.9%). The site of Gankette Balla in the Sahelian zone and Ndoffane in the Sudano-Guinean zone closed to river and vegetation, contributed particularly to the high density of *An. funestus* s.l. collected; (Annex A, Table A1).

Figure 4: *Anopheles* Species Composition collected across the country using HLC, PSC and ORC From May through December 2021

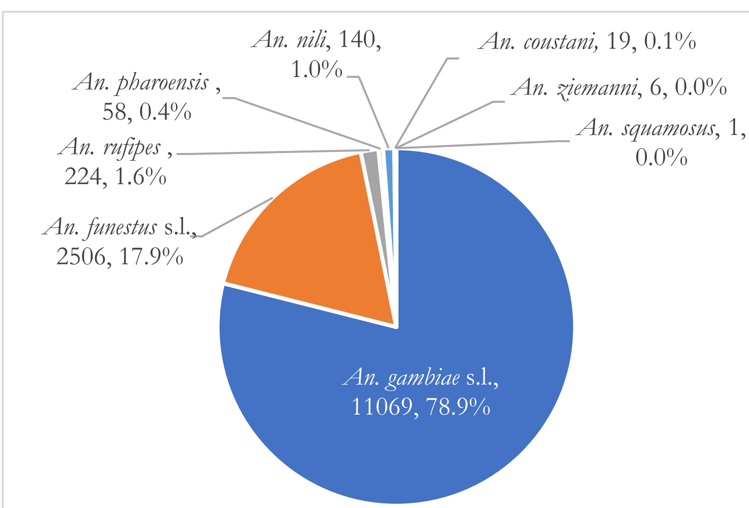
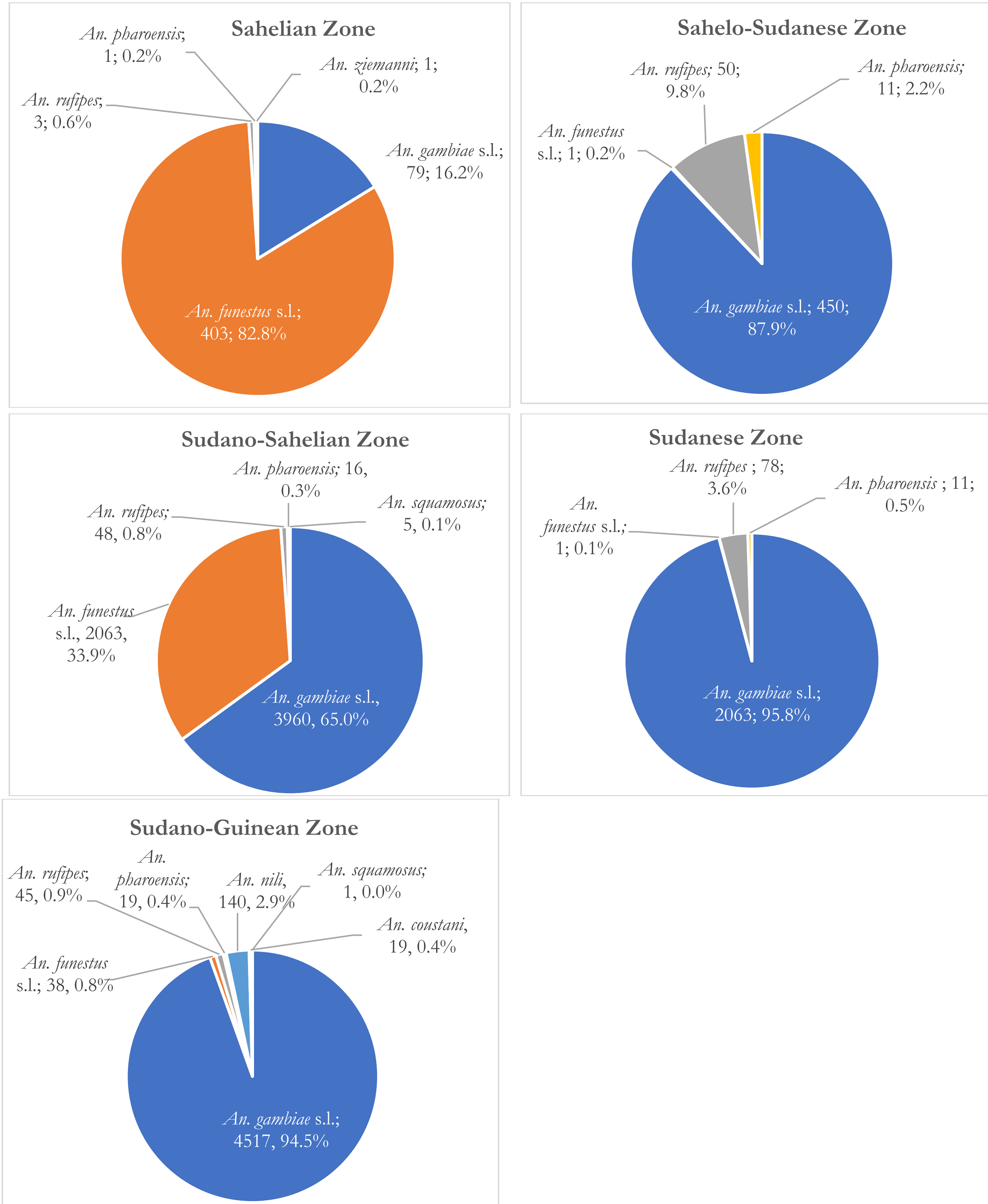


Figure 5: *Anopheles* Species Composition by Geographical Zone Collected Using HLC, PSC and ORC From May through December 2021

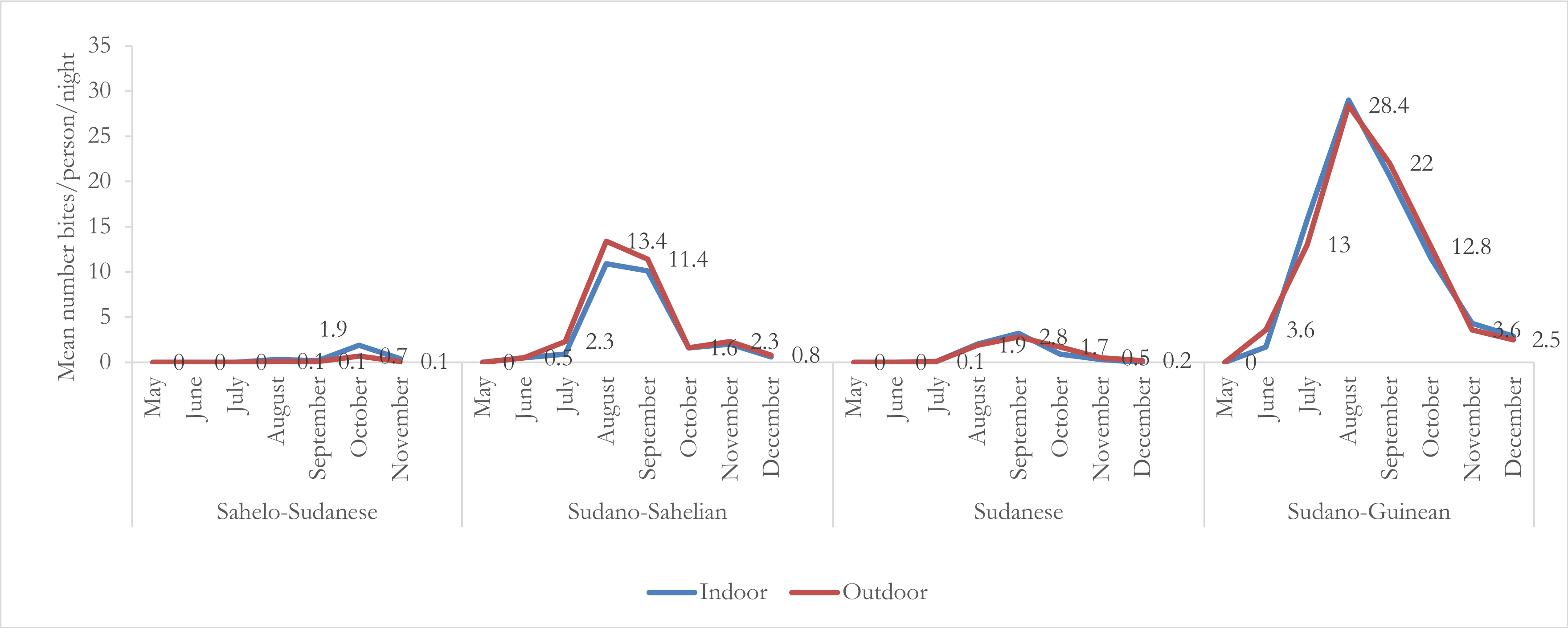


3.1.1.2 Human Biting Rate and Vector Biting Behavior By Geographical zone

3.1.1.2.1 Human Biting Rate Of An. gambiae s.l.

A total of 7,171 *An. gambiae* s.l. were collected using HLC at all sites. In all geographical zones, the average biting rate was estimated at 3.02 b/p/n. HBRs were highest in the Sudano-Guinean (9.12 b/p/n) and Sudano-Sahelian (3.70 b/p/n) zones. The lowest mean HBR (0.26 b/p/n) was recorded in the Sahelo-Sudanese zone (Annex 1, Table 2). The biting cycle was recorded in four of the zones surveyed, as the collection in the Sahelian zone as only PSCs were conducted. In these four zones, the indoor biting rates were similar to outdoor biting rates in Sudanese and Sudano-Guinean zones, but they were higher in Sahelo-Sudanese zone and lower in Sudano-Sahelian zone (Figure 5). Overall, the highest HBRs were recorded between August and September within all geographical areas except in Sahelo-Sudanese zone where the highest HBRs were recorded in October (Figure 6), coinciding with the rainy season within the country.

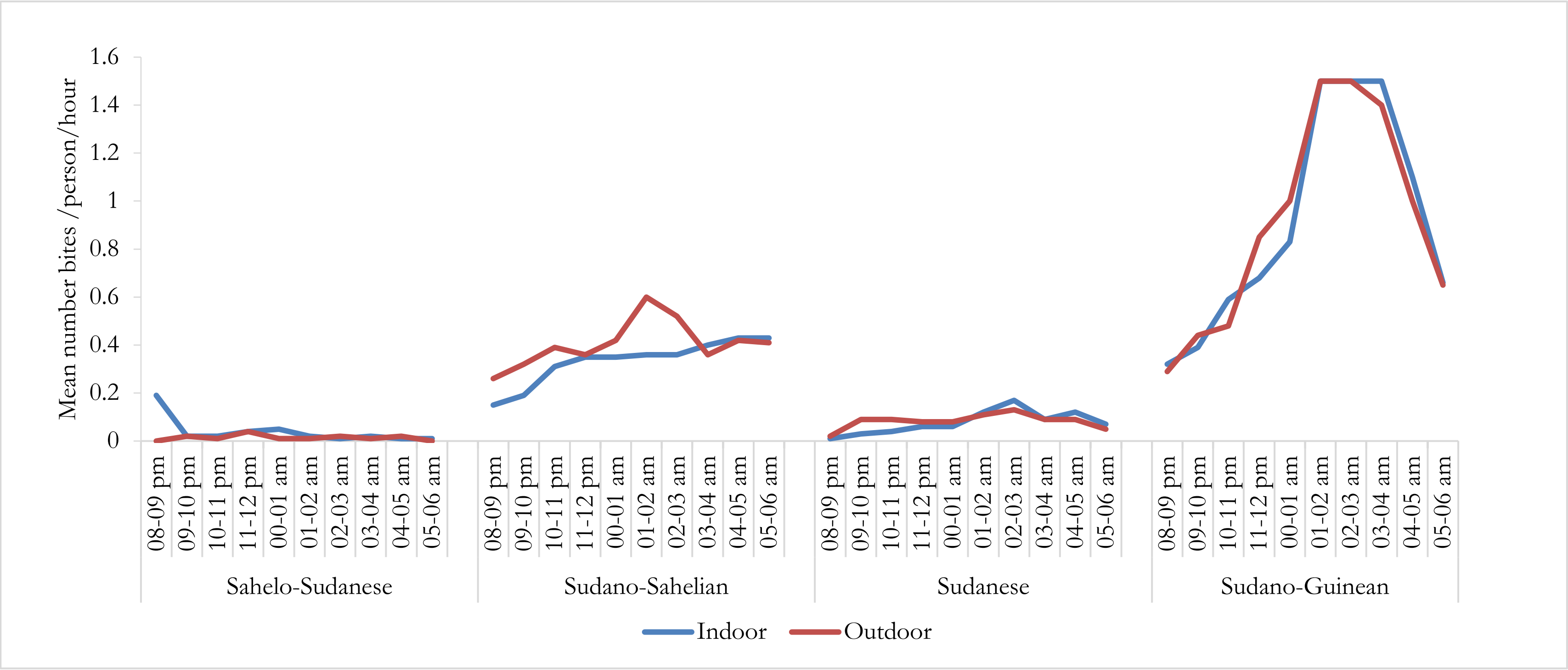
Figure 6: Monthly variation of *An. gambiae* s.l. Biting Rate by Geographical Zone



3.1.1.2.2 Biting Time of An. gambiae s.l.

The HLCs were conducted from 8 p.m. to 6 a.m. in the four geographical zones sampled. Except in the Sahelo-Sudanese zone where the mean hourly biting started rising from 11 p.m to 01 a.m. in the other zones it started increasing during the second half of the night (from 00h a.m) both indoors and outdoors. The highest peak hourly biting was recorded between 1 and 3 a.m in Sudano-Guinean zone. Furthermore, the mean hourly biting rates were higher both indoors and outdoors in the Sudano-Guinean zone, with about 1.5 bites/person/hour occurring between 2 and 3 a.m. In contrast, the other zones recorded less than 1 bite/person/hour in the Sudano-Sahelian zone and less than 0.2 bite/person/hour in the Sahelo-Sudanese and Sudanese zones, throughout the night, both indoors and outdoors (Figure 7).

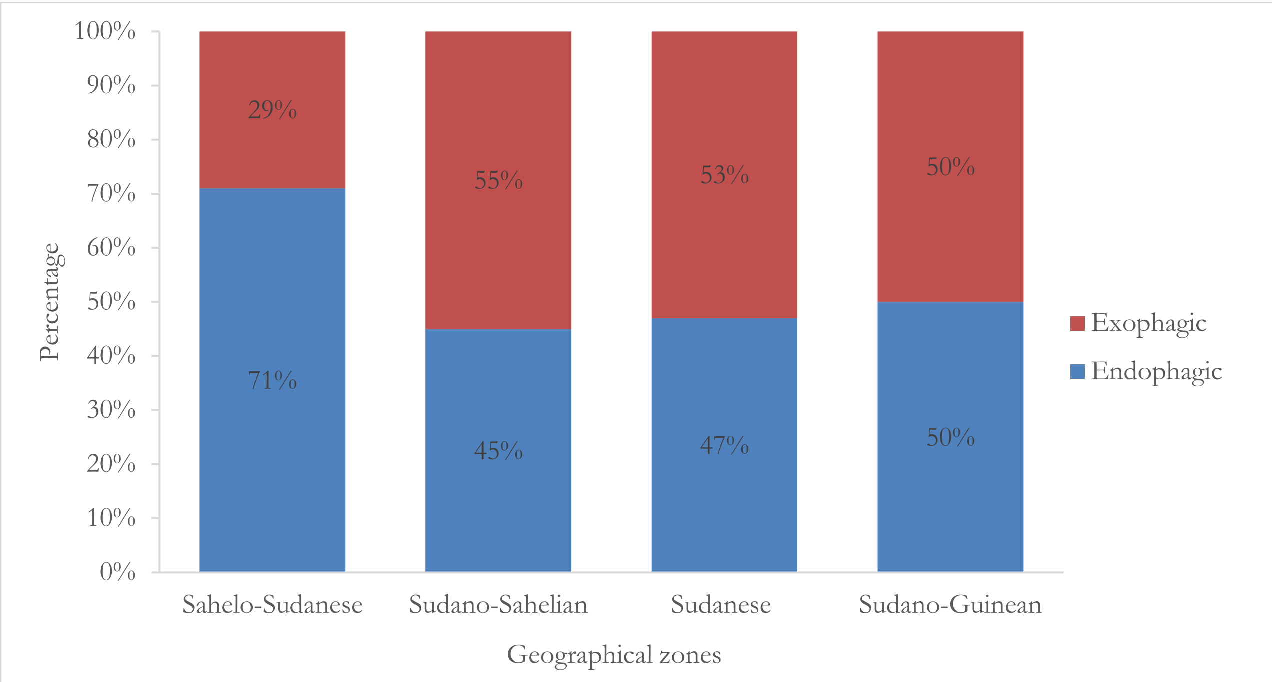
Figure 7: Mean Hourly Biting Rate of *An. gambiae* s.l. by Geographical Zone



3.1.1.2.3 Anopheles gambiae s.l. Endophagic Rate

The mean endophagic rate of all surveyed sites within all geographical zones was estimated to 0.49 (Annex 3), showing slightly higher outdoor biting by *An. gambiae* s.l. females overall (p <0.001). However, the vectors bite equally indoors and outdoors in the Sudano-Guinean and Sudanese zones and slightly higher indoor in the Sahelo-Sudanese zone (71% indoor) (Figure 8).

Figure 8: Endophagic Rate of *An. gambiae* s.l. by Geographical Zone

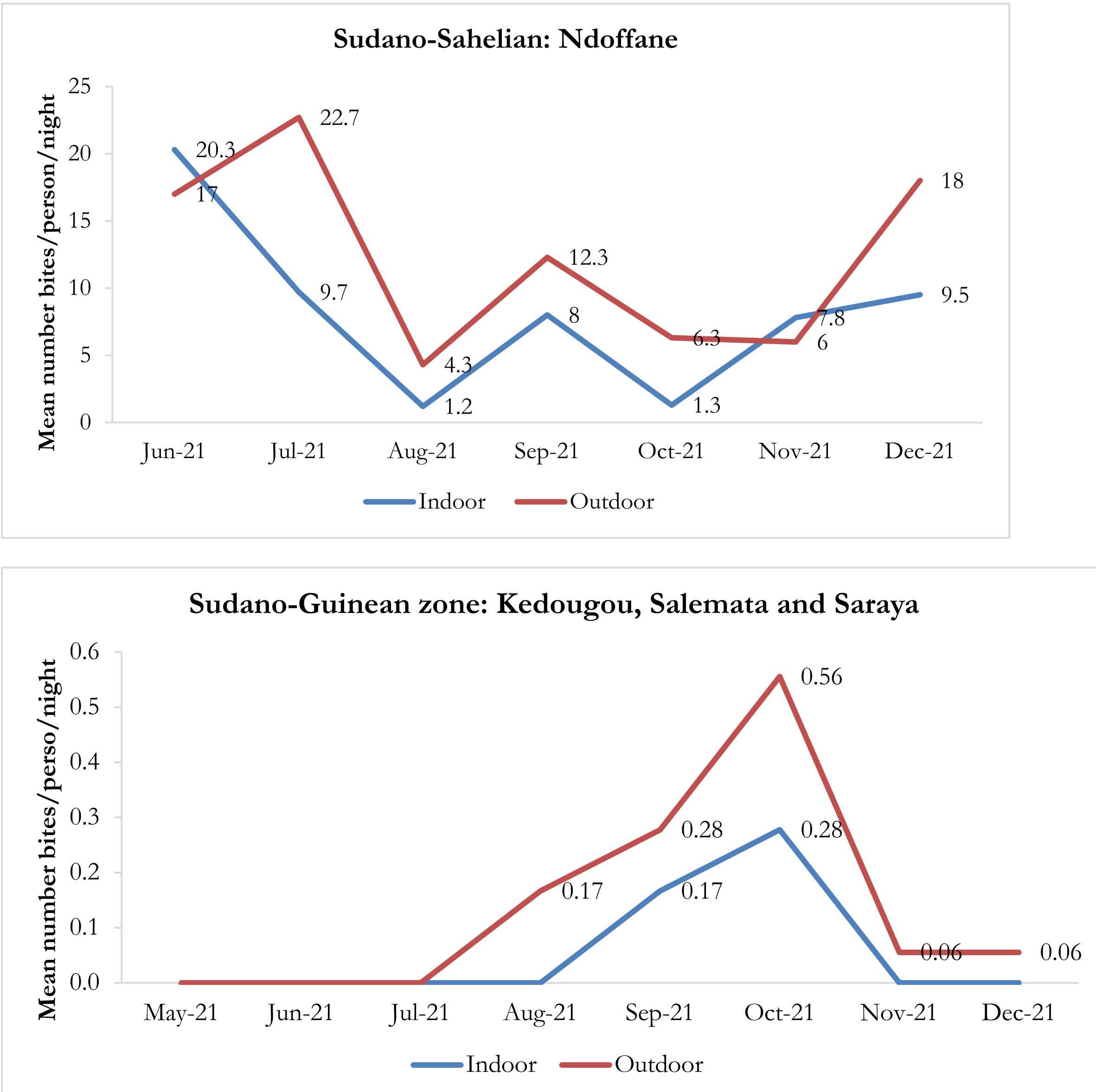


3.1.1.2.4 Anopheles funestus s.l. Human Biting rate

The mean HBR of *An. funestus* s.l. was < 1 b/p/n in all the sentinel sites, except in Ndoffane (13.65 b/p/n), in the Sudano-Sahelian zone, where most of the *An. funestus* s.l. (96.8% n= 867) was collected.

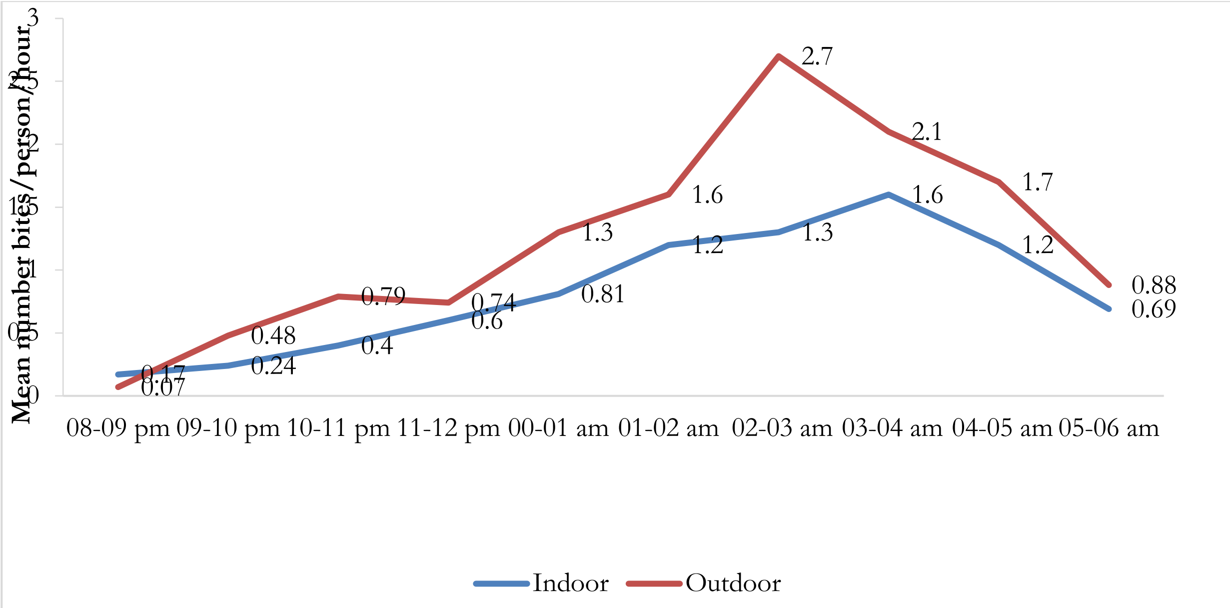
The remaining *An. funestus* s.l. (3.2%) collected using HLCs were found in the Sudano-Sahelian and Sudano-Sudanese zones (Annex 4), which included one specimen collected in Koungheul (Sudano-Sahelian zone) and 28 other specimens in Kédougou, Saraya, and Salémata (Sudano-Guinean zone). Because the majority of *An. funestus* s.l. were collected in only two geographical zones, the HBRs were estimated only for these areas. Very low (< 1 b/p/n) monthly biting rates were recorded in the Sudano-Guinean zone, with peak biting in October 2021 both indoor and outdoor. In contrast, in Ndoffane (Sudano-Sahelian zone), a peak of >22.7 b/p/n was observed in July 2021, outdoors. In both zones, *An. funestus* s.l. bites more outdoors than indoors most of the time (Figure 9).

Figure 9: Monthly variation of *An. funestus* s.l. Biting Rate Collected in The Sudan-Sahelian and Sudano-Guinean Zone



Peak *An. funestus* s.l. hourly biting occurred between 2:00 a.m. and 3:00 a.m. both outdoors (2.7 b/p/n) and 3:00 a.m. and 4:00 a.m. indoors (1.6 b/p/n) in Ndoffane the Sudano-Sahelian zone (Figure 10). Too, few mosquitoes (28) were collected in the Sudano-Guinean zone to determine peak biting.

Figure 10: Mean Hourly Biting rate of *An. funestus* s.l. in Ndoffane (Sudano-Sahelian Zone)



3.1.1.2.5 Anopheles funestus s.l. Endophagic rate

Anopheles funestus s.l. biting behavior was estimated only in Ndoffane (Sudano-Sahelian) where larger number of the vector was collected. Overall, *An. funestus* s.l. bites more outdoors (59% exophagic rate) than indoors (41% endophagic rate)

3.1.1.3 Indoor Resting Density and Abdominal Status Of Female Vectors By Geographical zone Collected Using PSCs

3.1.1.3.1 Anopheles gambiae s.l.

The mean IRD, as expressed by the mean number of female *An. gambiae* s.l. per room (f/r) was on average 1.4 f/r for the whole monitoring period. The highest IRDs were recorded in Sudano-Sahelian (2.7 f/r) zones, while the lowest IRDs were recorded in the Sudano-Guinean (0.6 f/r). (Figure 11; Annex A, Table A3)

The proportion of blood-fed females found inside houses was significantly lower in the Sudanese zone (40.4% of the total collected in the Sudanese zone) (p=0.001) when compared with in other zones (Figure 10). The highest proportion of blood-fed females was recorded in the Sudano-Guinean zone (75.7%) of the total collected, followed by the Sahelian zone (69.6%) (Figure 12).

Figure 11: Overall Monthly Indoor Resting Density of *An. gambiae* s.l. by Geographical Zone

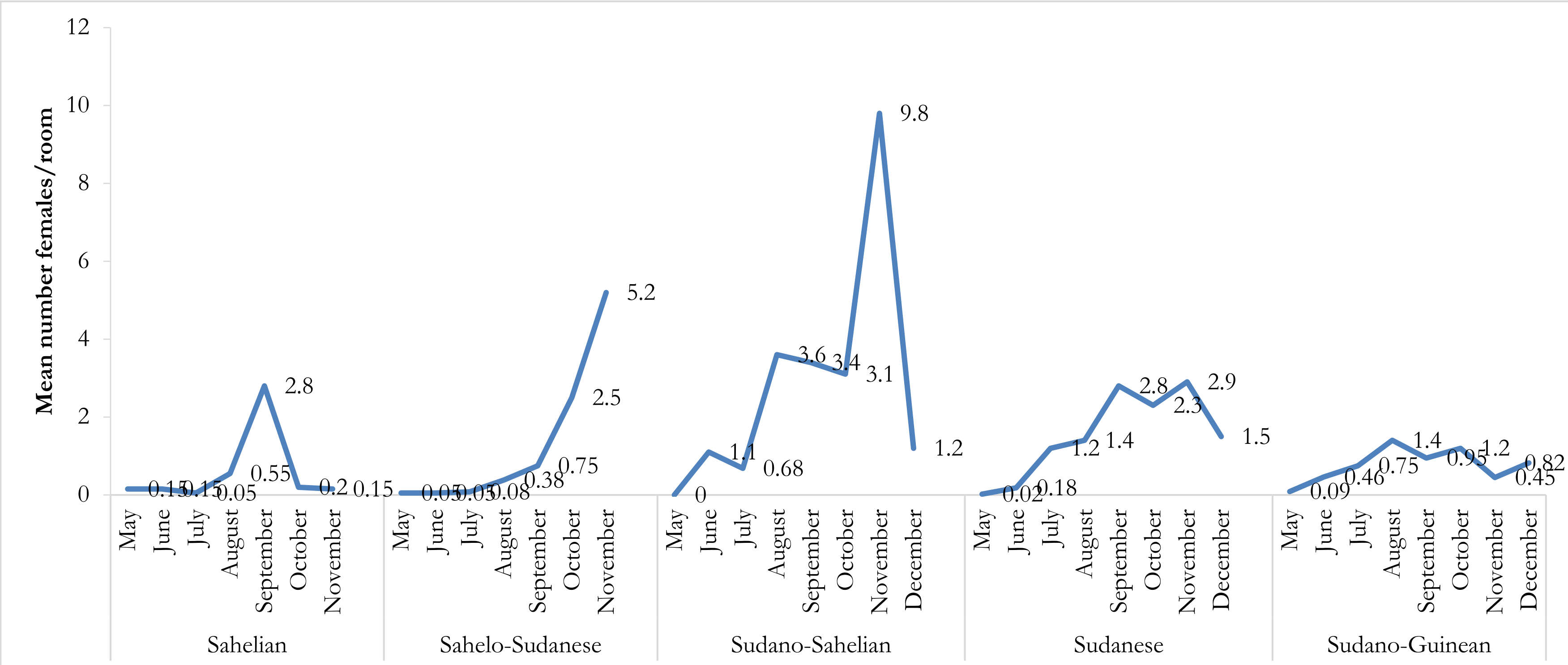
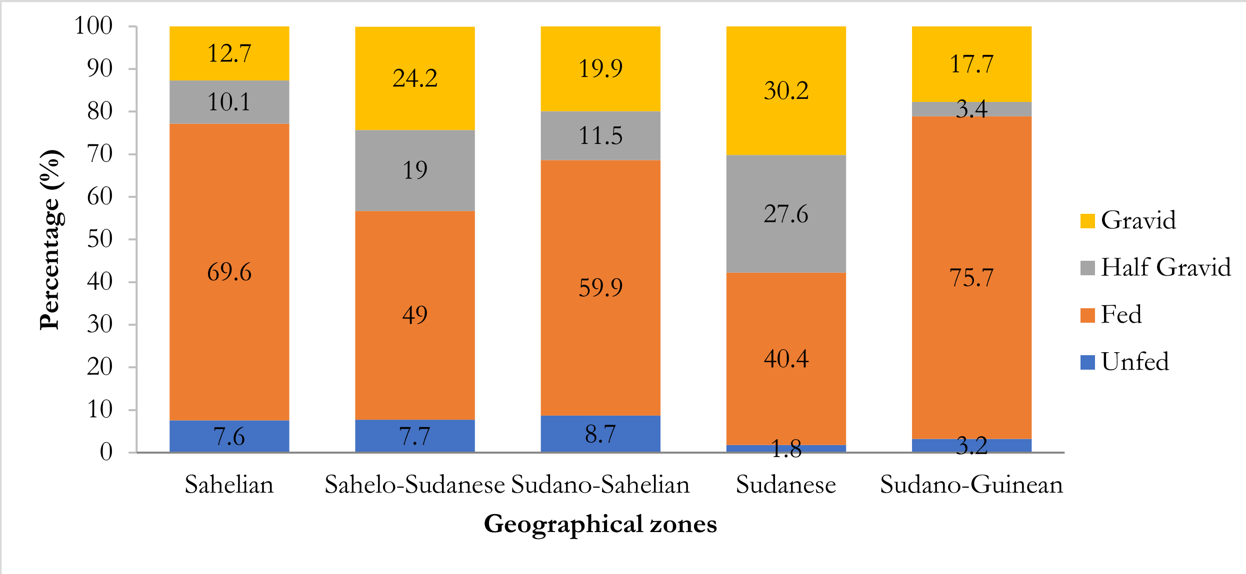


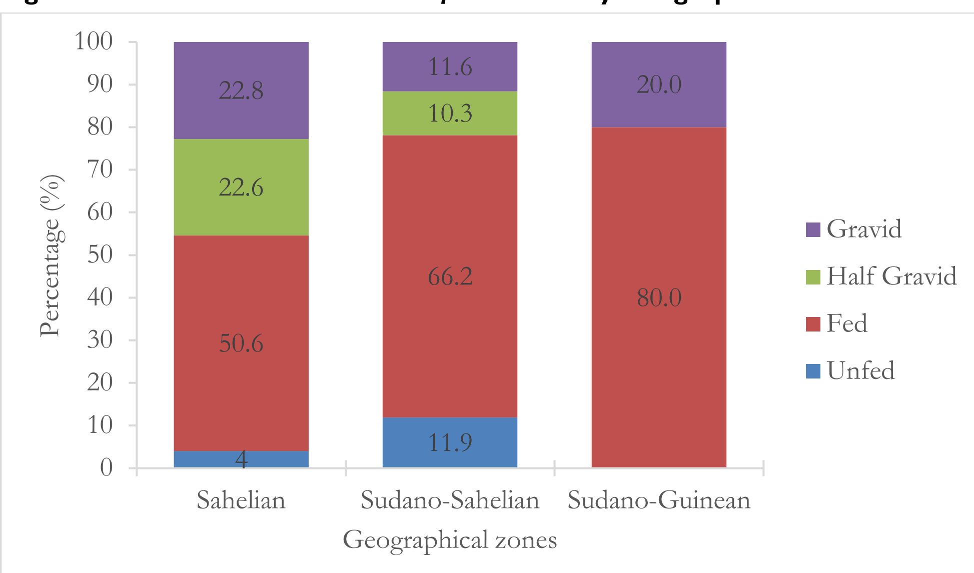
Figure 12: Abdominal Status of Female *An. gambiae* s.l. by Geographical Zone



3.1.1.3.2 ANOPHELES FUNESTUS S.L.

The average proportions of blood-fed *An. funestus* s.l. females were higher in Sudano-Guinean zone (80%) followed by the Sudano-Sahelian zone 66.2% (34/49) and Sahelian zone 50.6% (Figure 13). These are the zones where the majority of the *An. funestus* s.l. were collected.

Figure 13: Abdominal Status of *An. funestus* s.l. by Geographical Zone



3.1.1.1 Outdoor Resting Density and Abdominal Status Of Female Vectors Collected Using Prokopack in Tambacounda district

Compared to PSC, few *Anopheles* were collected outdoors through Prokopack aspiration in the four sentinel sites of Tambacounda district where PBO nets were distributed (Table 6). A total of 20 *An. gambiae* s.l. and 1 *An. rufipes* were collected.

Table 6: Abdominal status of *An. gambiae* s.l. collected outdoor with Prokopack (May to December 2021)

Village	Total Collected	Fed	Unfed	Gravid	Half gravid
Kouthia Farindella	2	1	0	1	0
Velingara Sabaké	0	0	0	0	0
Oundoundou	8	3	1	0	4
Safalou 1	10	6	0	3	1
Total	**20**	**10 (50%)**	**1 (5%)**	**4 (20%)**	**5 (25%)**

3.1.1.2 Parity Rate

The highest parity rates were recorded in the Sahelo-Sudanese zones (86%), while the largest number of mosquitoes dissected was observed in the Sudano-Guinean area (3276). *An. funestus* s.l. parity rate was relatively low in Ndoffane (Table 7)

Table 7: *An. gambiae* s.l. and *An. funestus* s. l. Parity Rate by Geographical Zone

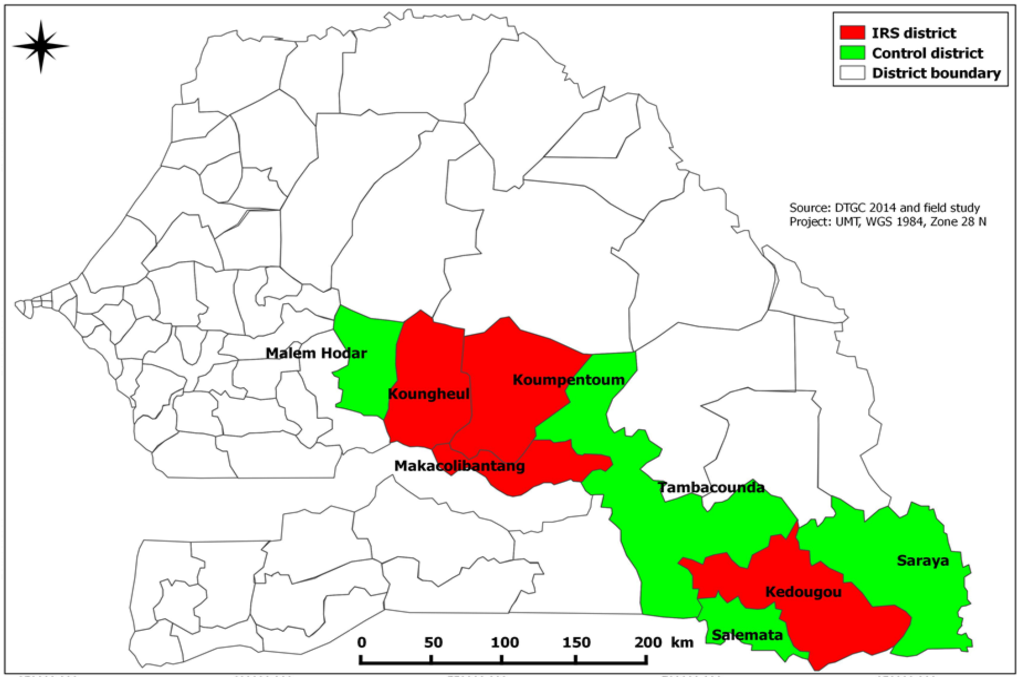
Geographical zone	*An. gambiae* s.l.		
	#Dissected	#Parous	Parity (%)
Sahelo-Sudanese	42	36	85.7
Sudano-Sahelian	767	301	39.2
Sudanese	551	242	43.9
Sudano-Guinean	3276	1912	58.4
Total	**4636**	**2491**	**53.7**
	An. funestus s.l.		
Sudano-Sahelian (Ndoffane)	514	171	33.3

3.2 Entomological Parameters Of IRS And Control Sites

3.2.1 IRS and Control Site Location

Longitudinal entomological monitoring was conducted in the four IRS sites (Kédougou, Koumpentoum, Koungheul, and Makacolibantang) and control sites (Saraya, Salémata, Tambacounda, and Malém Hodar) (Figure 14).

Figure 14: Map of the IRS and Control Sites

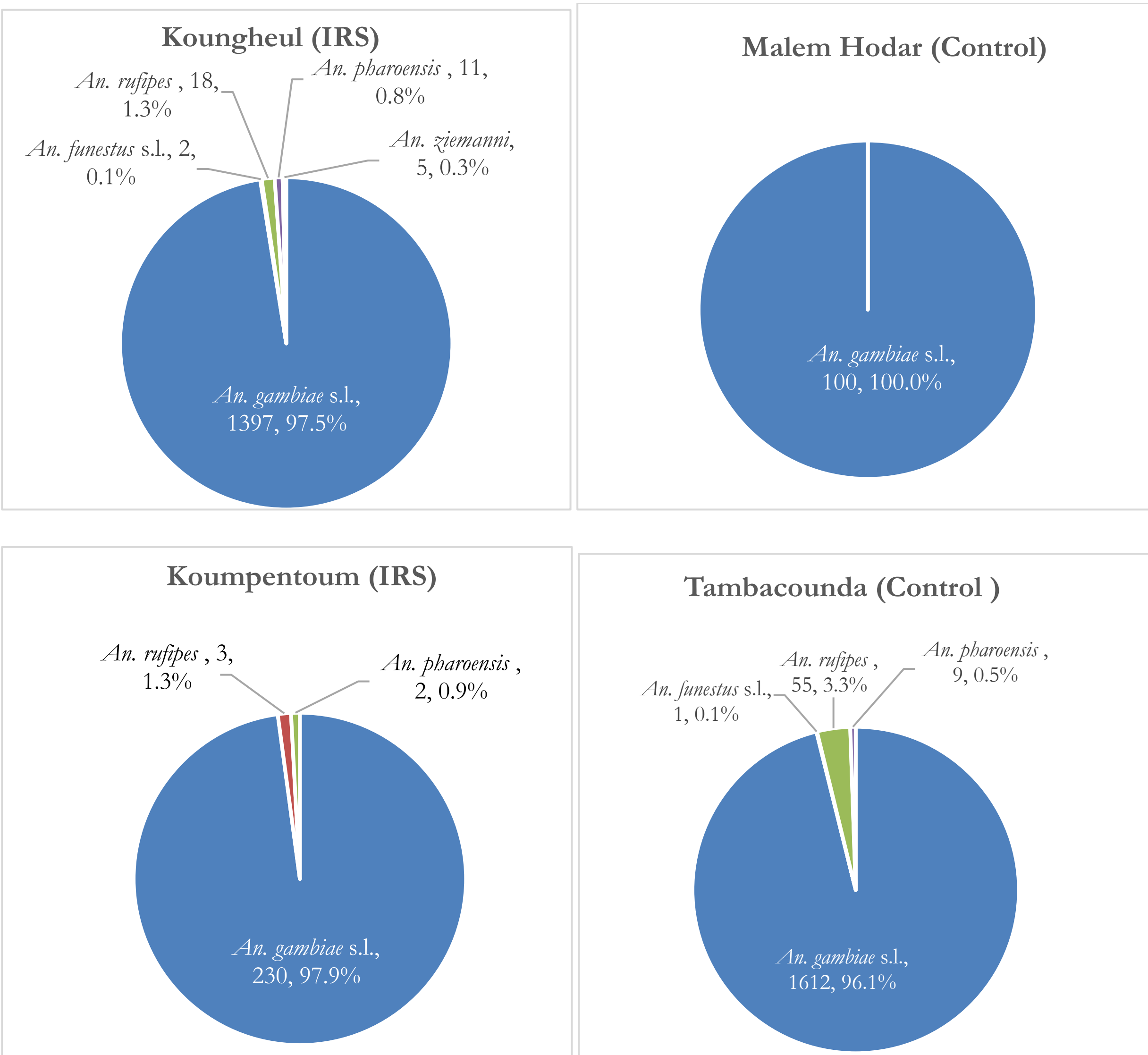


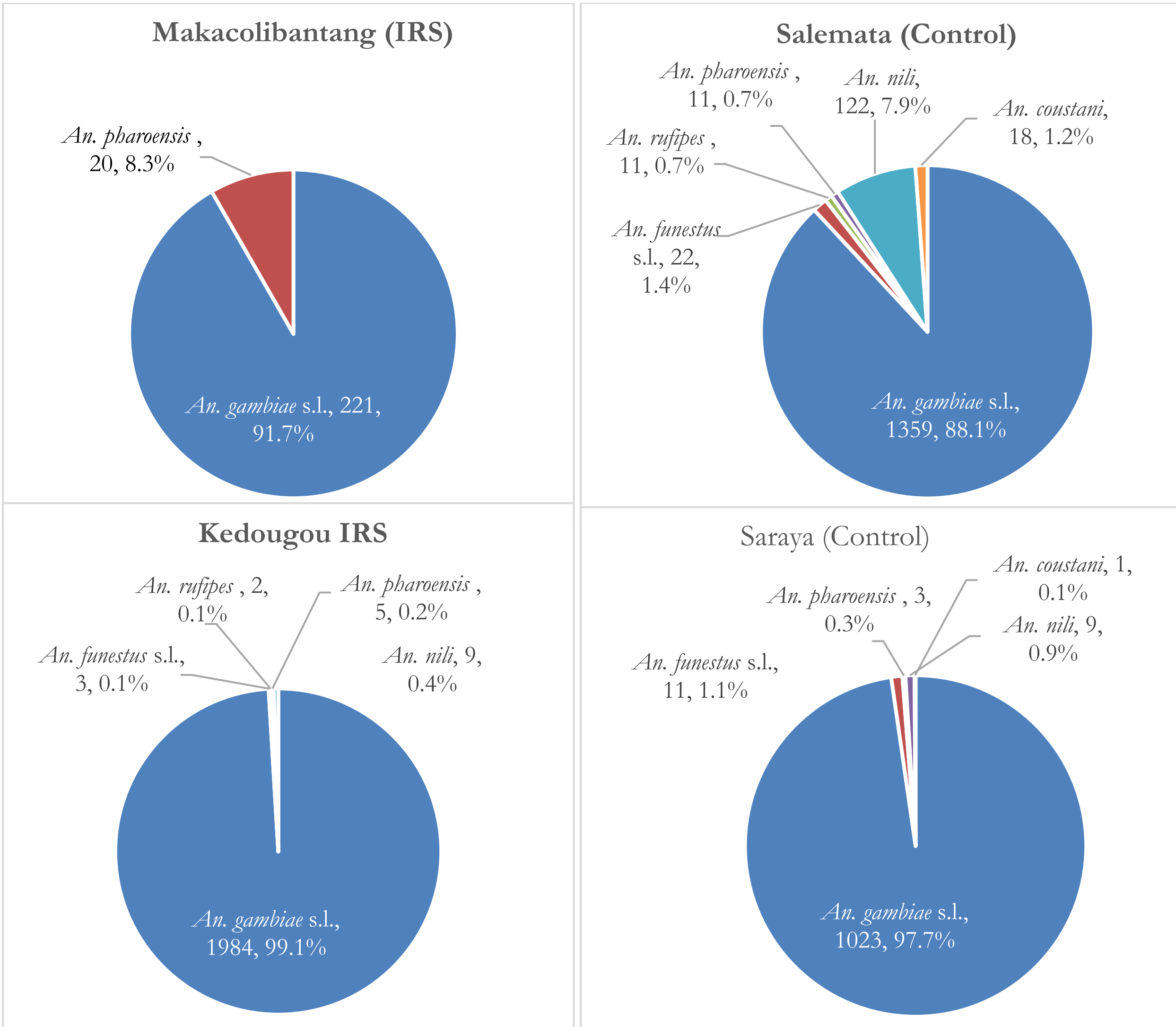
3.2.2 Species Composition

In all IRS sites, *An. gambiae* s.l. was the dominant vector, representing about 98% of collections done through HLCs and PSCs. *Anopheles rufipes* was also collected in all the four IRS districts and represented the second most common *Anopheles* species (1.1%), even though few were caught due to differences between sites. For example, Koungheul the IRS site is located in an area with adequate vector larval habitats with more retention of water while Malem Hodar is more sand area with less possibility of water to last longer on ground. More diversity of *Anopheles* was recorded in Kédougou, with six detected species (Figure 15, Annex A table A4).

As in the IRS districts, *An. gambiae* s.l. represented more than 94% of collections in control sites. *An. nili* was the second most common *Anopheles* species collected in Kédougou's control (3%). Larger numbers of *An. nili* were also collected in Salemata, with 8% (122/1543) of the total *Anopheles* collected in those sites (Figure 15).

Figure 15: *Anopheles* Species Composition in IRS Sites and Controls



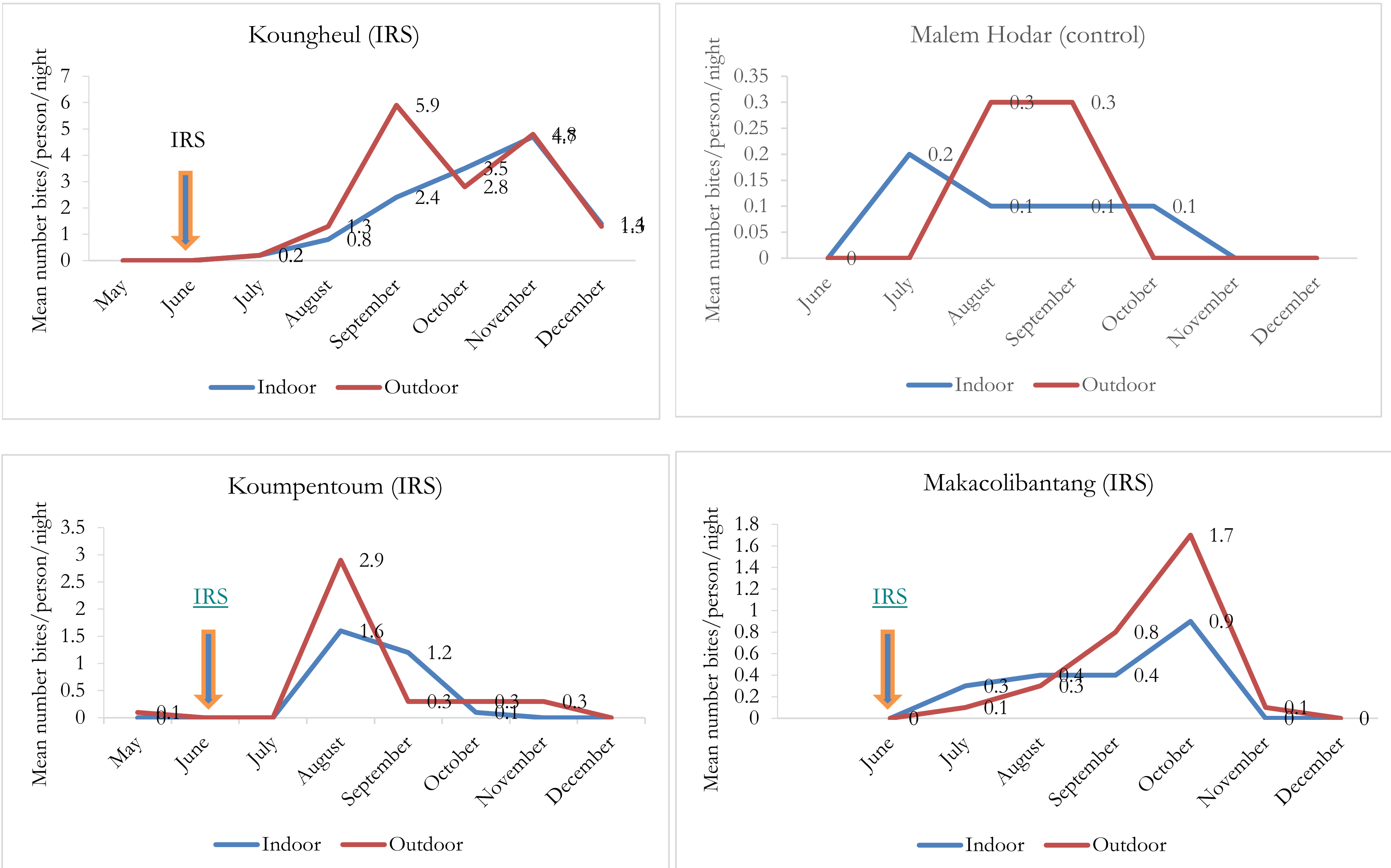


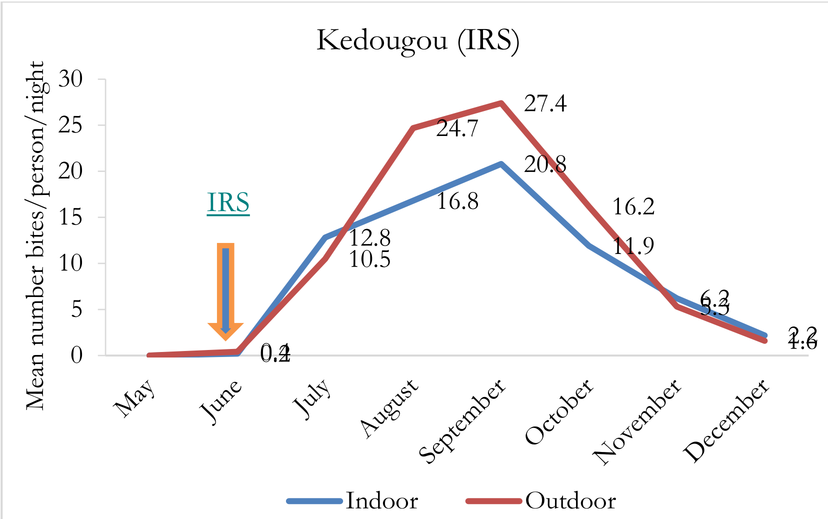
3.2.3 Human Biting Rate and Vector Behavior in IRS and Control Sites

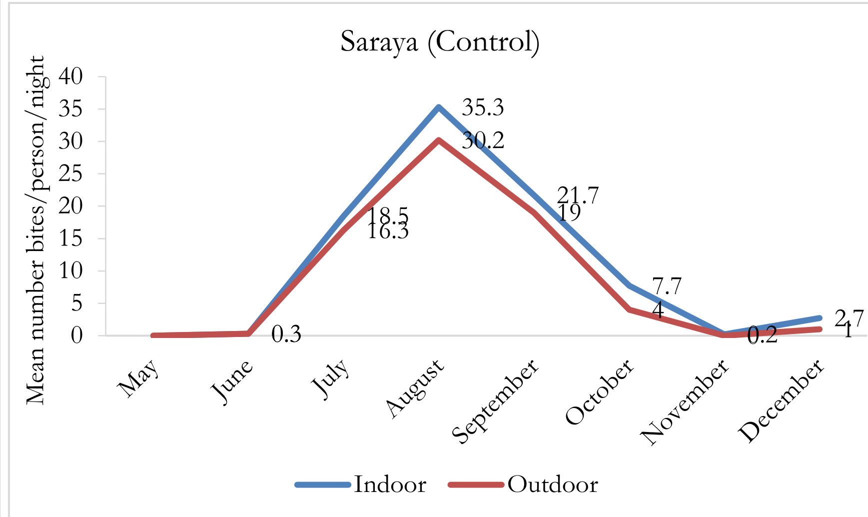
At all IRS sites, *An. gambiae* s.l. bite significantly more outdoors than indoors while in control sites indoor and outdoor biting rates were similar (Figure 21). The overall mean indoor and outdoor HBR in the Fludora Fusion sprayed sites of Kedougou was higher (< 4.9 b/p/n) than that recorded in the SumiShield sites (> 0.9 b/p/n) (Annex A; Table A5). The peak indoor/outdoor biting was recorded in August in Koumpentoum, in September in Kedougou and October in Makacolibantang. In Koungheul, peak outdoor biting was recorded in September and another peak of both indoor andoutdoor was observed in November. (Figure 6).

The HBR and behavior observed in the control districts showed that the indoor densities of the vectors were slightly higher than the outdoors, except in Malem Hodar, where an outdoor biting was observed from August to September. The overall mean indoor/outdoor HBR in Tambacounda, Saraya, and Salémata was above 14 b/p/n, and higher than that of Malem Hodar, which had less than 1.5 b/p/n both indoors and outdoors. The peak biting was also observed between August and October 2021 for the control sites (Figure 16).

Figure 16: Indoor and Outdoor HBR of *An. gambiae* s.l. in IRS Districts and their controls







3.2.4 *An. gambiae* s.l. Parity Rate in IRS and Control Sites

Parity rate gives information on the lifespan of the female population and represents a good indicator to estimate IRS impact. Lower parity rates suggest younger vector populations and therefore with individuals who are less likely to be infected. A total of 1,644 *An. gambiae* s.l. across IRS sites and 2,459, in controls sites were ovary-dissected for parity rates, of which respectively 61% and 54% were parous. The mean parity rates in both IRS sites and control sites were slightly high (Table 8), indicating that the vectors were old enough to transmit the sporozoite. However, only HLC collected mosquitoes were dissected for parity, including both from indoors and outdoors with similar parity rates (Table 8).

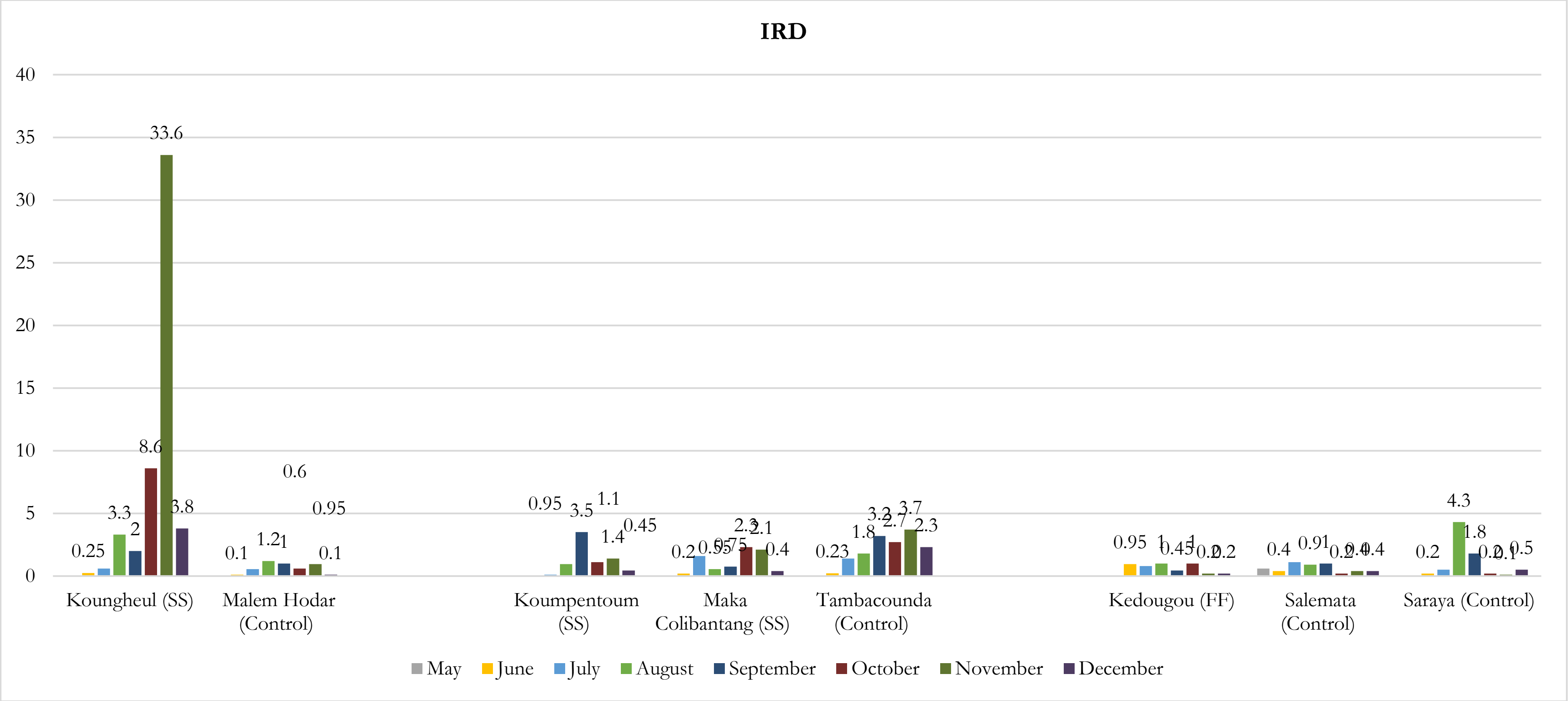
Table 8: Parity Rate of *An. gambiae* s.l. in IRS Districts and Controls

			Indoor				Outdoor				Total			
Insecticide	**Intervention**	**District**	**Collected**	**Dissected**	**Parous**	**% Parous**	**Collected**	**Dissected**	**Parous**	**% Parous**	**Collected**	**Dissected**	**Parous**	**% Parous**
Sumishield	IRS	Koumpentoum	34	12	9	75.0	47	8	6	75.0	81	20	15	75.0
	IRS	Koungheul	156	109	80	73.4	198	162	107	66.0	354	271	187	69.0
	IRS	Makacolibantang	24	15	11	73.3	36	17	11	64.7	60	32	22	69
	Subtotal		214	136	100	73.5	281	187	124	66.3	495	323	224	68.8
	Control	Tambacounda	334	246	100	40.7	339	253	105	41.5	673	499	205	41.1
	Control	Malem Hodar	5	2	1	50.0	6	4	0	0.0	11	6	1	16.7
	Subtotal		339	248	101	40.7	345	257	105	40.9	684	505	206	40.8
Fludora Fusion	IRS	Kedougou	853	662	416	62.8	1037	659	371	56.3	1890	1321	787	59.6
	Subtotal		853	662	416	62.8	1037	659	371	56.3	1890	1321	787	59.6
	Control	Salemata	698	622	381	61.3	604	527	309	58.6	1302	1149	690	60.0
	Control	Saraya	518	486	272	56.0	428	319	163	51.1	946	805	435	54.0
	Subtotal		1216	1108	653	58.9	1032	846	472	55.8	2248	1954	1125	57.6
Total IRS districts			1067	798	516	64.6	1318	846	495	58.5	2385	1644	1011	61.5
Total control districts			1555	1356	754	55.6	1377	1103	577	52.3	2932	2459	1331	54.1
Total IRS and controls			**2622**	**2154**	**1270**	**59.0**	**2695**	**1949**	**1072**	**55.0**	**5317**	**4103**	**2342**	**57.1**

3.2.5 Indoor Resting Density of An. gambiae s.l. in IRS Districts and Controls

A total of 1,290 houses were sprayed using PSCs in the SumiShield and control sites during the collection period, while 400 houses were visited in Kédougou and its controls (Saraya and Salémata). Koungheul recorded the highest IRD (5.2 f/r) among the IRS sites, while Tambacounda yielded the highest IRD (1.7 f/r) among the different control sites (Figure 17 and Annex A Table A6).

Figure 17: IRD *of An. gambiae* s.l. in IRS Districts and Controls



3.1.1.1 LABORATORY ANALYSIS

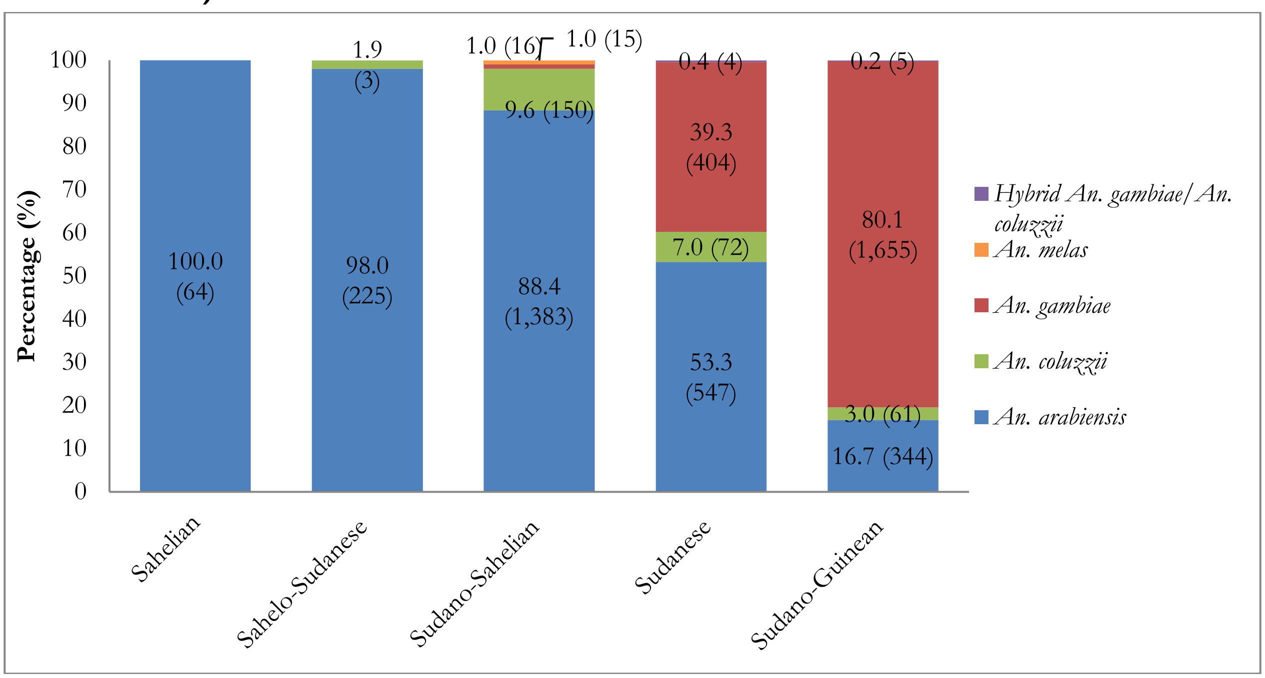
3.1.1.1.1 MOLECULAR SPECIES IDENTIFICATION

Species composition and spatial distribution of *An. gambiae* s.l.

A total of 4,948 *Anopheles gambiae* s.l. collected by human landing catch (3,227) and pyrethrum spray collections (1,721) were analyzed by PCR for species identification. The results revealed the presence of four species of the gambiae complex with An. *arabiensis* (51.8%, n = 2,563) being the predominant species, followed by *An. gambiae* (41.9%, n= 2,075). *Anopheles coluzzii* and *An. melas* were less represented with the respective proportion of 5.7% (n=286) and 0.4% (n= 15). Notably, few hybrids of *An. gambiae* and *An. coluzzii* (0.2%, n =9) were found in certain areas (Annex 1). Furthermore, only 37 of the selected samples did not amplify due to low or absence of DNA after checking with a Nanodrop.

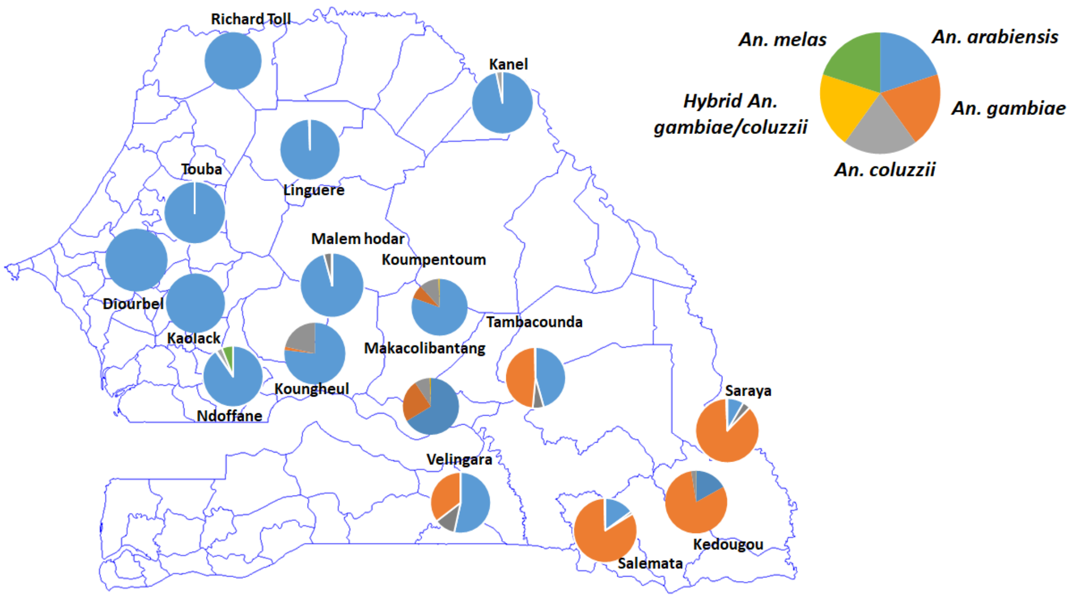
An. arabiensis was the most widespread species of the complex and predominated in almost all the surveyed biogeographical zones except in the Sudano-Guinean area, where *An. gambiae* was the most predominant species of the complex (Figures 17-18 & annex B table B1). *An. coluzzii* was more frequently detected in the Sudano-Sahelian and the Sudanese zones, in particular in the South-eastern part of the country where the hybrids of *An. gambiae/An. coluzzii* were also found. While the three species of the complex (*An. arabiensis, An. gambiae* and *An. coluzzii*) were found in sympatry in the central and southern parts of the country with greater diversity in the latter, *An. melas* was mainly confined to the Sudano-Sahelian zone notably in the district of Ndoffane (Figures 18-19 & Annex B table B2).

Figure 18: *An. gambiae* s.l. species composition and distribution by Geographical Zone (January to December 2021)



Numbers in bracket represent the number tested per species

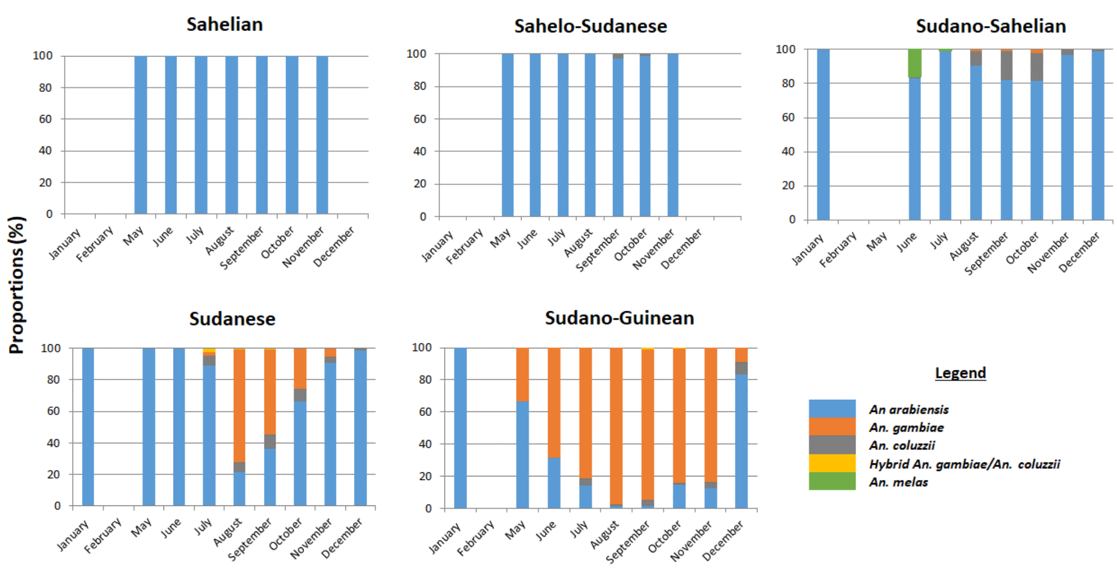
Figure 19: *An. gambiae* s.l. species composition and distribution in s entinel s ites (January to December 2021)



Temporal variation of *An. gambiae* s.l.

Over the survey period, *An. arabiensis* was the most frequent species collected in the Sahelian, Sahelo-Sudanese and Sudano-Sahelian zones year round, but during the dry season in the Sudanese and Sudano-Guinean zones, *An. gambiae* s.s. was the most frequent member of the complex collected during the rainy season, especially in the Sudano-Guinean districts (Figure 20 & AnnexB table B3). Notably, in all the biogeographical zones where it was found, *An. coluzzii* appeared only during the rainy season (between July and October), excepted in the Sahelian zone where it was absent.

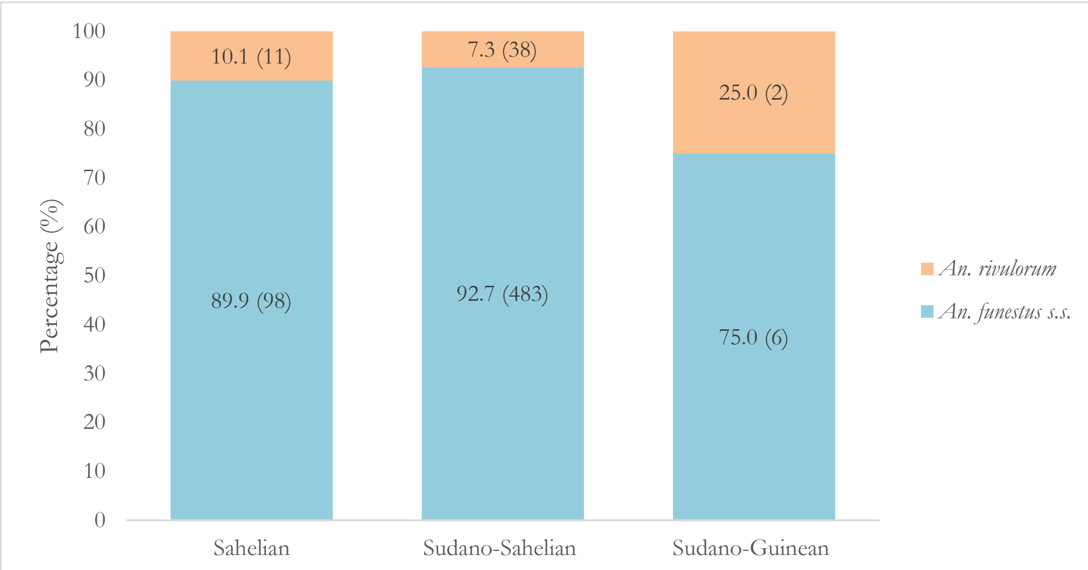
Figure 20: Monthly variation in the proportions of *An. gambiae* s.l. species by Geographical zones (January to December 2021)



Species composition of *An. funestus* s.l.

Molecular identification of the species of the *An. funestus* group showed that *An. funestus* s.s. (92.0%, n=588) was the predominant species of the group and found in the 4 geographical zones where *An. funestus* s.l. was collected. The second species of the group was identified as *An. rivulorum* representing 8% (n=51) of the group member recorded in 3 of the 4 sites (Figure 21).

Figure 21: *An. funestus* species composition and distribution by Geographical z one (January to December 2021)



3.1.1.1.2 Plasmodium falciparum Infection Rate

Infected females were found only in Sudanese and Sudano-Guinean zones among randomly selected *An. gambiae* s.l. from all zone sites. No infected female was detected out of the 366 mosquitoes analyzed in Sudano-Sahelian zone (Table 9). Overall, the infection index was similar between treated (IRS) respective control districts. Of the 19 specimens of *An. funestus* from the Sudano-Sahelian (1) and Sudano-Guinean (18) zones screened for presence of *P. falciparum* infection, One infected female out of18 was detected in the Sudano- Guinean zone. In the Non-IRS districts, infected *An. gambiae* s.l. were found only in the Sudano-Sahelian, Sudanese and Sudano-Guinean zones respectively in the sentinel sites of Touba (1/89), Tambacounda (1/294) and Velingara (1/143) (Table 10). Infected *An. funestus* s.l. were found in Richard toll in the Sahelian (0.5%, 2/360).

Table 9: *Plasmodium falciparum* Infection Rate in *An. gambiae* s.l. and *An. funestus* s.l. in IRS vs Control Districts

Geographical zone	District	*An. gambiae* s.l.				*An. funestus* s.l.			
		T	P	CSI	P-value	T	P	CSI	P-value
Sudano-Sahelian	IRS (Koungheul)	355	0	0	NS	1	0	0	NS
	Control (Malem hoddar)	11	0	0					
Sudanese	IRS (Koumpentoum)	30	1	0.033	P> 0.05 (NS)				
	Control (Koussanar)	68	1	0.014					
	IRS (Makacolibantang)	48	1	0.02	P> 0.05 (Ns)				
	Control (Lycounda)	143	2	0.014					
	IRS (Kédougou)	845	12	0.014	P> 0.05 (NS)				

Sudano-Guinean	Control (Saraya & Salemata)	934	19	0.02		18	1	0.06	NS

Table 10: *Plasmodium falciparum* Infection Rate of *An. gambiae* s.l. and *An. funestus* s.l. in Unsprayed Vector Surveillance Districts

Geographical zone	District	*An. gambiae* s.l.			*An. funestus* s.l.		
		T	P	CSI	T	P	CSI
Sahelian	Richard Toll	72	**0**	**0**	360	2	0.005
Sahelo-Sudanese	Kanel	23	0	0	0	0	0
	Linguere	33	0	0	0	0	0
Sudano-Sahelian	Diourbel	40	0	0	0	0	0
	Touba	89	1	0.011	0	0	0
	Kaolack	506	0	0	0	0	0
	Ndoffane	80	0	0	519	0	0
Sudanese	Tambacounda (PBO-LLINs-site) *	294	1	0.003	0	0	0
Sudano-Guinean	Velingara	143	1	0.007	1	0	0

T = Tested ; P= Posit   ive ; CSI = Circumsporozoite index

Of the 660 *An. arabiensis*, 1571 *An. gambiae* s.s., 144 *An. coluzzii* and 6 *An. coluzzii*/*An. gambiae* hybrid females screened for the presence of *P. falciparum*, the average infection rate was the highest for *An. coluzzii* (1.38%, [0.048 - 0.200]), followed by *An. gambiae* s.s. (1.9%, [0.014 - 0.034]), while *An. arabiensis* was the least infected member of the complex 0.45% (0.008 - 0.040). No *P. falciparum* (*P.f.*) infected specimen was found among the tested hybrids (Table 11). *Plasmodium falciparum* infected females were detected among the natural population of *An. gambiae* s.s. only in the Sudanese (2/52 in the internal control of the Makacolibantang IRS district) and Sudano-Guinean zones (10/696 and 18/781 respectively in IRS district of Kedougou and its control). The two *P.f.* infected *An. coluzzii* females were found in Koumpentoum (1/5) in the Sudanese zone, and in Kedougou (1/21) in the Sudano-Guinean zone. *Anopheles arabiensis* was found positive for Pf circumsporozoite in the control sites of Koumpentoum (1/54), and in the IRS sites of Makacolibantang (1/25) and Kedougou (1/122).

Table 11: *Plasmodium falciparum* infection rate of the *An. gambiae* s.l. species collected by HLC in the IRS-District and their control (January to December 2021)

Geographical zone	Sites status	*An. arabiensis*			*An. gambiae*			*An. coluzzii*			*Hybrid*		
		T	P	CSI	T	P	CSI	T	P	CSI	T	P	CSI
Sudano-sahelian	Koungheul (IRS)	250	0	0	6	0	0	68	0	0	-	-	-
	Control	8	0	0	1	0	0	1	0	0	-	-	-
Sudanese	Koumpentoum (IRS)	22	0	0	3	0	0	5	1	**0.2**	1	0	0
	Control	54	1	**0.02**	10	0	0	2	0	0	-	-	-
	Makacolibantang (IRS)	25	1	**0.04**	16	0	0	7	0	0	1	0	0

	Control	70	0	0	58	2	**0.03**	16	0	0	-	-	-
Sudano-guinean	Kedougou (IRS)	122	1	**0.01**	696	10	**0.01**	21	1	**0.05**	-	-	-
	Control (Saraya & Salemata)	109	0	0	781	18	**0.02**	24	0	0	4	0	0

3.1.1.1.3 Entomological Inoculation Rate

The Entomological Inoculation Rate (EIR) of *An. gambiae* s.l. populations varied according to the biogeographical zones (table 12). While no transmission was noted in the Sudano-Sahelian zone, relatively high EIR was estimated in the untreated control areas in the Sudanese and the Sudano-Guinean zones. Across the treated area, the EIR was the highest in the Sudano-Guinean zone, being 20 times higher in Kedougou (Sudano-Guinean) than in Makacoulibantang (Sudanese) (0.122/0.006) and 24.8 times higher in Kedougou than Koumpentoum (Sudanese) (0.122/0.005). This difference was less marked between the two treated districts of Makacoulibantang and Koumpentoum both located in the Sudanese zone. Indeed, the level of the transmission was about twice higher (0.006/0.005) in Makacoulibantang compared to Koumpentoum. Infected females of *An. funestus* were found only in the Sudano-Guinean zone with an EIR of 0.007 ib/p/n.

Table 12: *Plasmodium falciparum* infection rate and Entomological Inoculation Rate of *An. gambiae* s.l. and *An. funestus* s.l. in the IRS vs the controls districts

Geographical zone	District	*An. gambiae* s.l.			*An. funestus s.l.*		
		HBR	**CSI**	**EIR**	**HBR**	**CSI**	**EIR**
Sudano-Sahelian	Koungheul (IRS)	1.64	0	0	0	0	0
	Control (Malem hoddar)	0.05	0	0	0	0	0
Sudanese	Koumpentoum (IRS)	0.14	0.033	0.005	0	0	0
	Control (Koussanar)	1.42	0.015	0.021	0	0	0
	Makacolibantang (IRS)	0.31	0.0208	0.006	0	0	0
	Control (Lycounda)	4.03	0.014	0.056	0	0	0
Sudano-Guinean	Kedougou (IRS)	8.75	0.014	0.122	0	0	0
	Control (Saraya & Salemata)	10.42	0.02	0.208	0.12	0.056	0.007

At the other sentinel sites where IRS was not performed, infection was only found in *An. gambiae* s.l. females collected in the Sudanese zone in Tambacounda district with an EIR of 0.003 bi/h/n. No *An. funestus* females were found infected in these sites (Table 13).

Table 13: *Plasmodium falciparum* Infection Rate of *An. gambiae* s.l. and Entomological Inoculation Rate Collected by HLC in the Surveyed Sites

Geographical zone	District	*An. gambiae* s.l.			*An. funestus*		
		HBR	CSI	EIR	HBR	CSI	EIR
Sahelo-Sudanese	Kanel	0.12	0	0	0	0	0
	Linguere	0.17	0	0	0	0	0
Sudano-Sahelian	Kaolack	19.64	0	0	0	0	0
	Ndoffane	1.28	0	0	10.27	0	0
Sudanese	Tambacounda (PBO-LLIN site) *	0.91	0.003	0.003	0	0	0

In the Sudanese and Sudano-Guinean zones, malaria transmission is ensured by *An. arabiensis*, *An. gambiae* and *An. coluzzii* (Table 14). In the districts monitored in the Sahelo-Sudanese and Sudano-Sahelian zones, a lack of involvement in malaria transmission was noted for these three species despite their presence. The highest levels of infection recorded were obtained in *An. gambiae* females in the Sudano-Guinean zone with a maximum EIR of 0.103 ib/h/n in Salemata (Table 14).

Table 14: *Plasmodium falciparum* Infection Rate and Entomological Inoculation Rate of *An. gambiae* s.l. Species from IRS-Districts and Their Controls (January to December 2021)

Geographical zone	District	*An. arabiensis*			*An. gambiae*			*An. coluzzii*			*Hybrid An. gambiae/coluzzii*		
		HBR	CSI	EIR	HBR	CSI	EIR	HBR	CSI	EIR	HBR	CSI	EIR
Sudano-Sahelian	Koungheul	1.157	0	0	0.028	0	0	0.315	0	0	0	0	0
	Control	0.037	0	0	0.005	0	0	0.005	0	0	0	0	0
Sudanese	Koumpentoum	0.102	0	0	0.014	0	0	0.023	0.2	0.01	0.005	0	0
	Control	0.563	0.02	0.01	0.104	0	0	0.021	0	0	0	0	0
	Makacolibantang	0.141	0.04	0.01	0.12	0	0	0.042	0	0	0.005	0	0
	Control	0.729	0	0	0.604	0.035	0.02	0.167	0	0	0	0	0
	Tambacounda (PBO-LLINs-site)	0.086	0	0	0.343	0.006	0	0.009	0	0	0.002	0	0
Sudano-Guinean	Kedougou	0.565	0.01	0	3.245	0.014	0.05	0.097	0.05	0.01	0	0	0
	Control	0.505	0	0	3.648	0.023	0.08	0.111	0	0	0.019	0	0
	Saraya	0.269	0	0	2.907	0.022	0.07	0.148	0	0	0.019	0	0
	Salemata	0.741	0	0	4.389	0.024	0.1	0.074	0	0	0.019	0	0

3.1.1.1.4 KDR EAST (L1014S) AND WEST (L1014F) MUTATIONS

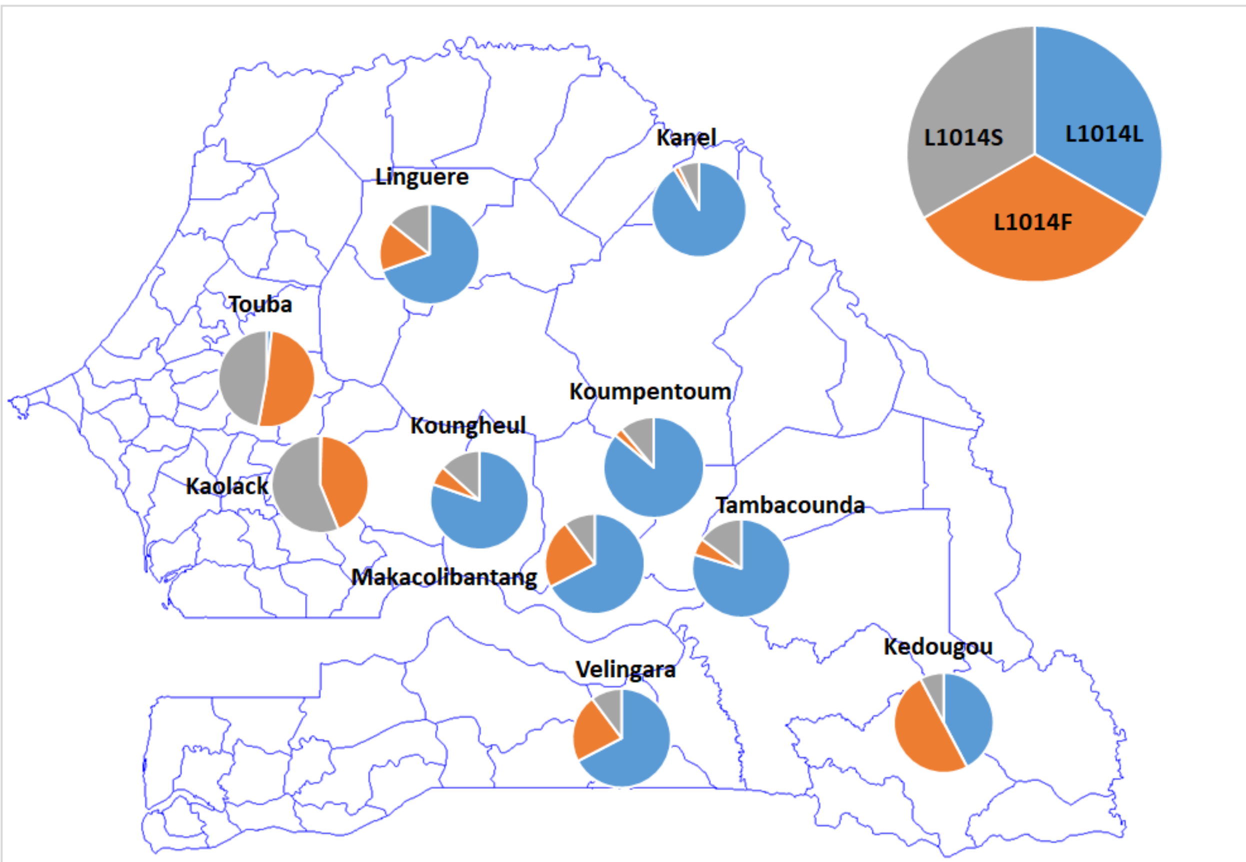
Genotypic and Allelic Frequencies

The mutations L1014S and L1014F, responsible for cross-resistance to pyrethroids and organochlorines, were investigated in the different *Anopheles gambiae* s.l populations that had been exposed to insecticides. Genotyping results revealed the presence of both mutations in all the sentinel districts (Figure 22 and Table 15).

The frequency of recorded Kdr mutations was higher in the urban areas of Touba and Kaolack with a predominance of the 1014S allele in Kaolack (56%: 109/194) and the 1014F allele in Touba (51%: 91/ 178).

In the north and center of the country, a low presence of Kdr-east and Kdr-west mutations was observed with a predominance of the sensitive allele (L1014L). In addition, a higher proportion of kdr mutations was noted in the south-eastern area of the country with a marked presence of the L1014F allele in Kedougou (Figure 22).

Figure 22 : Distribution of allelic frequencies of KDR mutations in sentinel districts



L1014S = *Kdr*-east resistant allele., L1014F = *Kdr* -west resistant allele, L1014L = Susceptible allele

Table 15: Genotype and allelic frequencies of *Kdr*-west and *kdr*-east mutations in *An. gambiae* s.l. by districts

Districts	Total	Genotype (%)						Allele (%)			
		SS	SRw	SRe	RwRw	RwRe	ReRe	Total	S	Rw	Re
Kanel	57	48 (84.21)	0	8 (14.03)	1 (1.75)	0	0	114	104 (91.22)	2 (1.75)	8 (7.02)
Linguère	56	37 (66.07)	1 (1.78)	3 (5.36)	8 (14.28)	1 (1.78)	6 (10.71)	112	78 (69.64)	18 (16.07)	16 (14.28)
Touba	89	0	0	3 (3.37)	32 (35.95)	27 (30.34)	27 (30.34)	178	3 (1.68)	91 (51.12)	84 (47.19)
Kaolack	97	0	0	1 (1.03)	17 (17.52)	50 (51.55)	29 (29.90)	194	1 (0.51)	84 (43.30)	109 (56.18)
Koungheul	71	52 (73.24)	2 (2.82)	8 (11.27)	3 (4.22)	1 (1.41)	5 (7.04)	142	114 (80.28)	9 (6.34)	19 (13.38)
Koumpentoum	90	69 (76.67)	3 (3.33)	14 (15.56)	0 (0)	2 (2.22)	2 (2.22)	180	155 (86.11)	5 (2.78)	20 (11.11)
Tambacounda	54	39 (72.22)	1 (1.85)	7 (12.96)	2 (3.70)	1 (1.85)	4 (7.41)	108	86 (79.63)	6 (5.56)	16 (14.81)
Makacolibantang	69	39 (56.52)	5 (7.25)	10 (14.49)	12 (17.39)	2 (2.90)	1 (1.45)	138	93 (67.39)	31 (22.46)	14 (10.14)
Kedougou	58	20 (34.49)	2 (3.45)	7 (12.07)	28 (48.28)	0 (0)	1 (1.72)	116	49 (42.24)	58 (50)	9 (7.75)
Velingara	49	30 (61.22)	1 (2.04)	5 (10.20)	10 (20.41)	1 (2.04)	2 (4.08)	98	66 (67.34)	22 (22.44)	10 (10.20)

Genotype frequency of *Kdr* mutations according to the mosquito phenotypic status (Surviving vs Dead)

The genotype data, presented in the Table 16, revealed that the two kdr mutations were found among dead and live mosquitoes post-exposure to insecticides. A significant difference (P<0.05) in the frequency of *kdr* mutations between dead and alive mosquitoes was observed in the districts of Kanel, Linguere, Tambacounda, Kedougou and Vélingara. But were similar in the remaining districts (P >0.05). This suggests that other underlying resistance mechanisms than the target site mutations such as the metabolic mechanisms are likely involved in the phenotypic resistance observed among the study populations. It therefore becomes critical to carry out additional studies to fully understand all the putative mechanisms underlying the phenotypic resistance observed in these populations.

Table 16: Genotypic prevalence of Kdr-west and kdr-east mutations according to the phenotypic status of *An. gambiae* s.l. females after being exposed to insecticides

Districts	Status	Genotype							P-value	Allele			
		N	SS	SRw	SRe	RwRw	RwRe	ReRe		N	S	Rw	Re
Kanel	Dead	25	18	0	6	1	0	0	*P < 0.05*	50	42	2	6
	Surviving	32	30	0	2	0	0	0		64	62	0	2
Linguere	Dead	40	30	0	3	5	0	2		80	63	10	7
	Surviving	16	7	1	0	3	1	4	*P < 0.05*	32	15	8	9
Touba	Dead	27	0	0	2	10	6	9		54	2	26	26
	Surviving	62	0	0	1	22	21	18	P > 0.05	124	1	65	58
Kaolack	Dead	28	0	0	1	3	13	11	P > 0.05	56	1	19	36
	Surviving	69	0	0	0	14	37	18		138	0	65	73
Koungheul	Dead	59	43	1	7	2	1	5		118	94	6	18
	Surviving	12	9	1	1	1	0	0	P > 0.05	24	20	3	1
Koumpentoum	Dead	73	55	3	11	0	2	2		146	124	5	17
	Surviving	17	14	0	3	0	0	0	P > 0.05	34	31	0	3
Tambacounda	Dead	36	30	0	2	1	1	2	*P < 0.05*	72	62	3	7
	Surviving	18	9	1	5	1	0	2		36	24	3	9
Makacolibantang	Dead	35	22	2	5	5	1	0		70	51	13	6
	Surviving	34	17	3	5	7	1	1	P > 0.05	68	42	18	8
Kedougou	Dead	35	17	2	7	8	0	1		70	43	18	9
	Surviving	23	3	0	0	20	0	0	*P < 0.05*	46	6	40	0
Velingara	Dead	32	22	1	5	2	0	2	*P < 0.05*	64	50	5	9
	Surviving	17	8	0	0	8	1	0		34	16	17	1

N = Number tested; RR, RS and SS represent the different genotypes with R corresponding to the resistant mutant allele (either for the kdr-west or kdr-east) and S to the susceptible wild type allele. P-value determined between dead and live mosquitoes tested

Genotypic Prevalence of *kdr* mutations by species

Genotyping of the species of the *Anopheles gambiae* complex performed (Table 17) showed the presence of both mutations kdr-west and kdr-east in all the three identified species namely *An. arabiensis, An. gambiae* and *An. coluzzii.*

Table 17: Genotype prevalence of the *kdr*-west and *kdr*-east mutations by species and tested sites

Districts	Species	Genotype							P-value	Allele			
		N	SS	SRw	SRe	RwRw	RwRe	ReRe		N	S	Rw	Re
Kanel	*An. arabiensis*	57	48	0	8	1	0	0	NA	114	104	2	8
Linguere	*An. arabiensis*	56	37	1	3	8	1	6	NA	112	78	18	16
Touba	*An. arabiensis*	89	0	0	3	32	27	27	NA	178	3	91	84
Kaolack	*An. arabiensis*	97	0	0	1	17	50	29	NA	194	1	84	109
Koungheul	*An. arabiensis*	71	52	2	8	3	1	5	NA	142	114	9	19
Koumpentoum	*An. arabiensis*	90	69	3	14	0	2	2	NA	180	155	5	20
Tambacounda	*An. arabiensis*	51	37	1	7	1	1	4	P > 0.05	102	82	4	16
	An. gambiae	3	2	0	0	1	0	0		6	4	2	0
Makacolibantang	*An. arabiensis*	61	38	5	10	5	2	1	***P < 0.05***	122	91	17	14
	An. coluzzii	1	1	0	0	0	0	0		2	2	0	0
	An. gambiae	7	0	0	0	7	0	0		14	0	14	0
Kedougou	*An. arabiensis*	40	20	2	7	10	0	1	***P < 0.05***	80	49	22	9
	An. gambiae	18	0	0	0	18	0	0		36	0	36	0
Velingara	*An. arabiensis*	43	30	1	5	4	1	2	***P < 0.05***	86	66	10	10
	An. coluzzii	1	0	0	0	1	0	0		2	0	2	0
	An. gambiae	4	0	0	0	4	0	0		8	0	8	0
	Hybrid *An. gambiae/An. coluzzii*	1	0	0	0	1	0	0		2	0	2	0

N = Number tested; RR, RS and SS represent the different genotypes with R corresponding to the resistant mutant allele (either for the Vgsc-1014F or Vgsc-1014S) and S to the susceptible wild allele Vgsc-1014L, NA = Not Applicable

At the species level, *An. arabiensis* displayed the highest prevalence of homozygous (RR) and heterozygous (RS) resistant genotypes for both *kdr* mutations, especially in the urban districts of Touba and Kaolack. Nevertheless, in the Southern part of the country (Sudanese and Sudano-Guinean), *An. gambiae* recorded the highest prevalence of homozygote RR.

3.1.1.1.5 3.1.1.1.5 ACE-1 R MUTATION

Investigation of the *Ace*-1R (G119S) mutation conferring carbamates and organophosphates cross-resistance was investigated from a sub-sample of 481 *An. gambiae* s.l. (Table 18). Of the 481 tested, 41 failed to amplify (8.5%).

The results revealed that there was not Ace 1R gene within the population tested.

Table 18: Genotype frequencies for the *Ace*-1R mutation in *An. gambiae* s.l. by tested sites

Districts	N	Genotypes		
		SS	RS	RR
Kanel	45	43	0	0
Linguere	50	43	0	0
Touba	49	48	0	0
Kaolack	49	48	0	0
Koungheul	50	44	0	0
Koumpentoum	48	46	0	0
Tambacounda	45	36	0	0
Makacolibantang	50	46	0	0
Velingara	47	41	0	0
Kedougou	48	45	0	0
Total	481	440	0	0

3.3 WALL BIOASSAYS

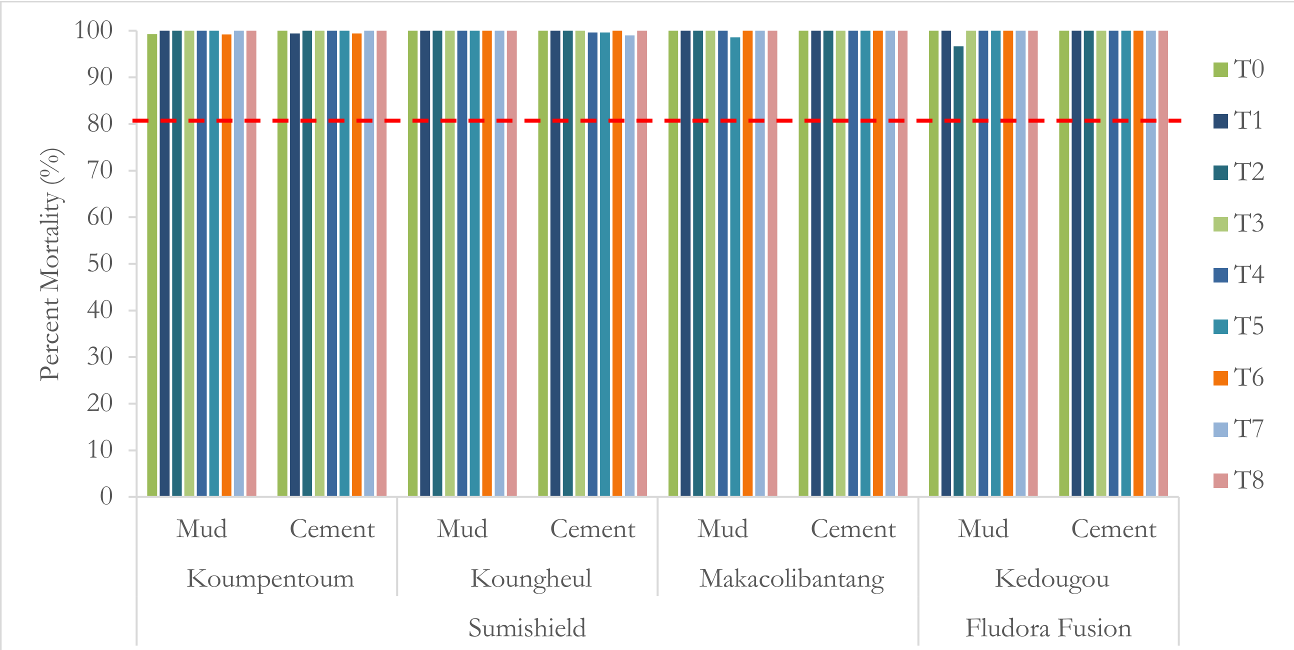
3.3.1 IRS SPRAY QUALITY ASSURANCE

A mortality rate of 99 to 100% on susceptible laboratory-reared *An. coluzzii* was recorded on all tested cement and mud walls in all IRS sites. The quality of the treatment at the sites sprayed with Fludora Fusion and SumiShield was therefore considered to be adequate.

3.3.2 MONTHLY INSECTICIDE DECAY RATE BY IRS DISTRICTS

The residual efficacy of treatments was evaluated during eight months in the four IRS districts (July 2021 to February 2022) (Figure 23). The results indicate that both insecticides showed a high residual efficacy with an average mortality rate above 99%, on all wall types tested (mud and cement; Figure 23).

Figure 23: Monthly Cone Bioassay Mortality Rates of The Susceptibility Laboratory *An. coluzzii* Strain on SumiShield and Fludora Fusion Sprayed on Mud and Cement by IRS District (*Red line represents the 80% efficacy threshold each bar represents % mortality up to five days over successive months)*



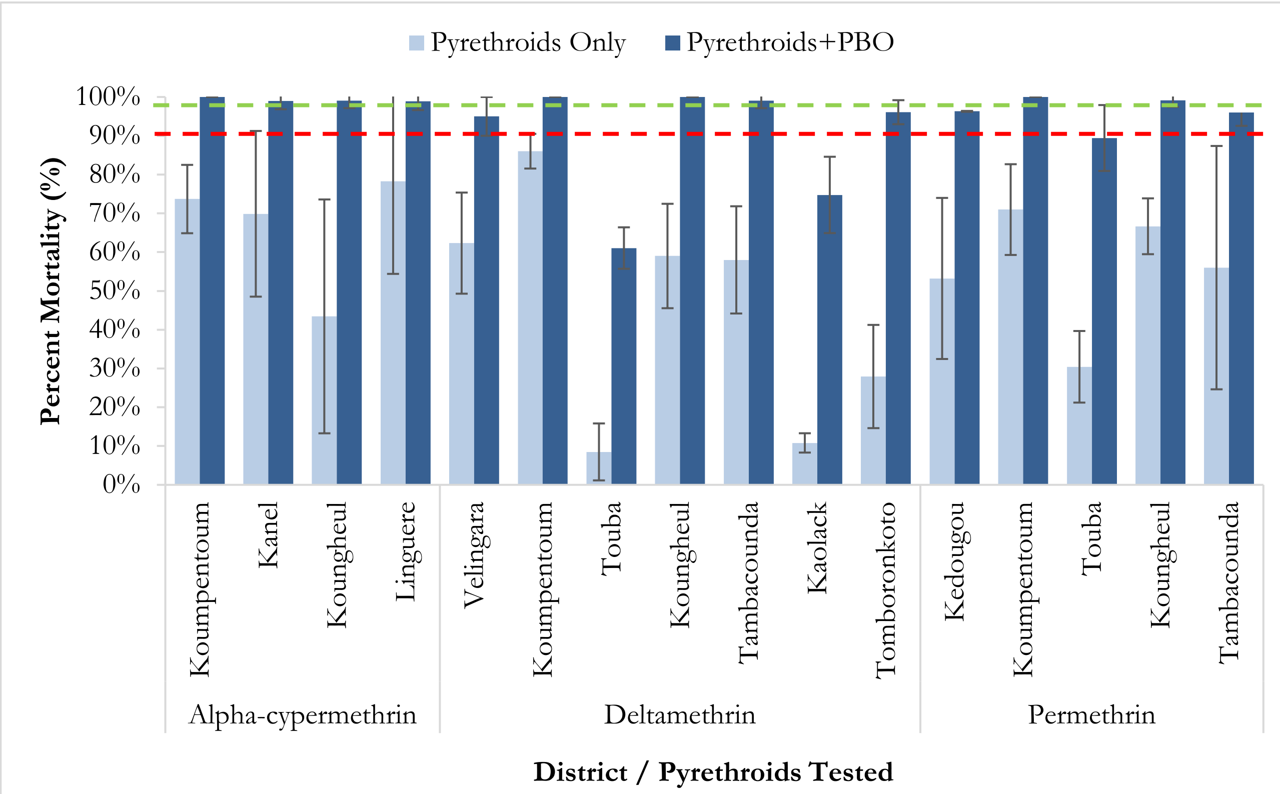
3.4 Malaria Vector Susceptibility To Insecticides

WHO insecticide susceptibility tests were carried out only against *An. gambiae* s.l., the main vector species collected in all the surveyed sites. CDC bottle assays were also conducted in selected sites, using chlorfenapyr and clothianidin insecticides. All the sites could not be surveyed, and all insecticides were not tested at all sites, due to delay in delivery of impregnated paper. The paper insecticides susceptibility testing was conducted late in October to December, when the rain was reduced, the chance to collect enough larvae for testing limited. Annex B, Table B11 presents the sites where insecticide susceptibility testing was carried out.

3.4.1 Susceptibility and Synergist Assays

The synergist assay test was conducted in nine sentinel districts including IRS and piperonyl butoxide (PBO) net distribution areas (Figure 24). With deltamethrin, pre-exposure to PBO reversed the resistance status of the *An. gambiae* s.l. population of Koungheul, Koumpentoum and Tambacounda to susceptibility, while a partial increment of mortality was recorded in Touba, Kaolack, Velingara and Kédougou. Piperonyl butoxide + permethrin tested in five of the sites also reversed the resistance status of the *An. gambiae* s.l. population of Koungheul, and Koumpentoum and yielded a partial mortality increment in Touba, Tambacounda and Kédougou. With alpha cypermethrin tested in four sentinel districts, pre-exposure to PBO reversed the resistance status of the *An. gambiae* s.l. population of Koungheul, Koumpentoum and Kanel and Linguere (Figure 24).

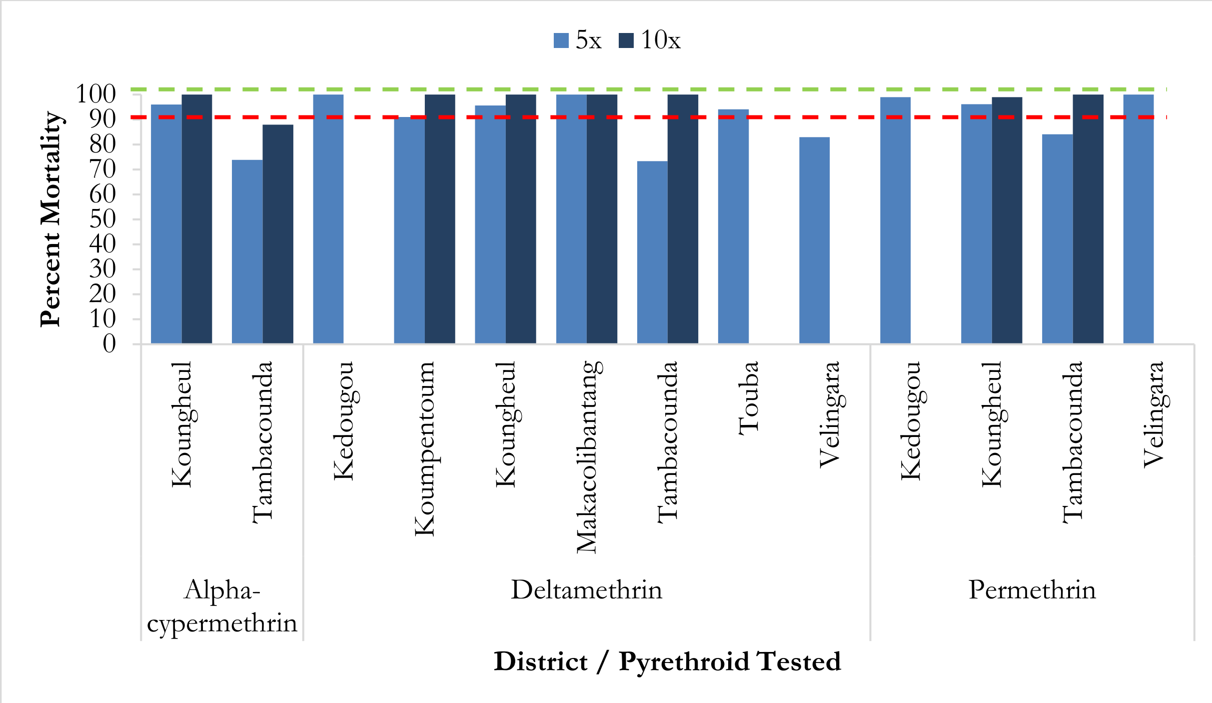
Figure 24: Mortality Rate of *An. gambiae* s.l. Against Deltamethrin, Permethrin and Alpha-cypermethrin After Pre-Exposure to piperonyl Butoxide



3.4.2 Intensity Of Resistance To Pyrethroids

Pyrethroid resistance intensity testing was done in 07 out of 16 sites. Populations tested were moderately resistant for deltamethrin in 6/7 sites, for permethrin at all 4 sites tested and for alpha cypermethrin in one of the two sites where the test was conducted. High resistant was found for deltamethrin in Velingara and for alpha cypermethrin in Tambacounda. (Figure 25)

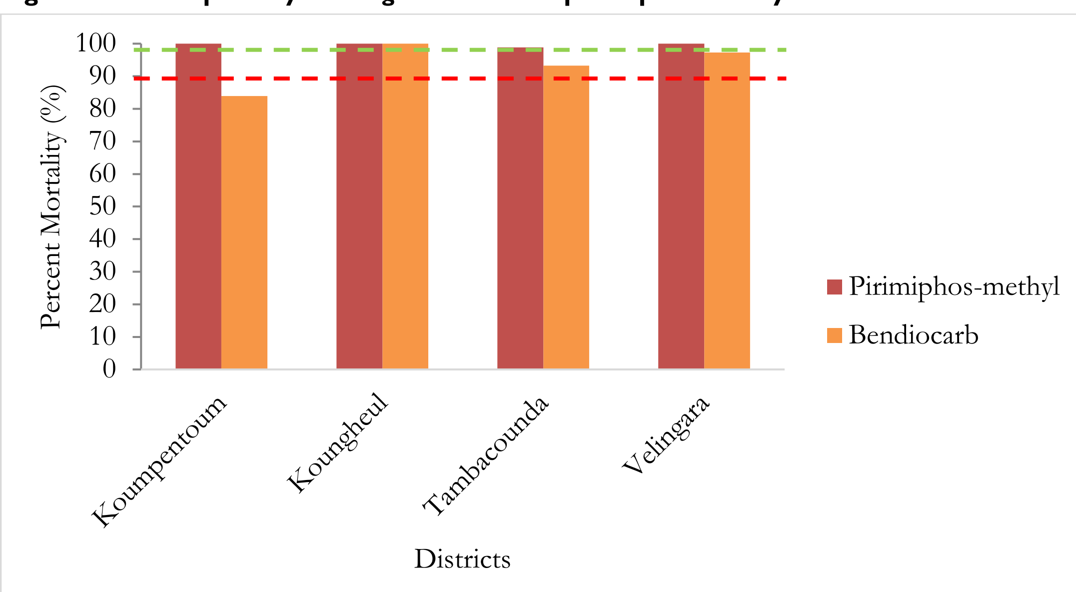
Figure 25: Resistance Intensity of *An. gambiae* s.l. By Sites Surveyed



3.4.3 Susceptibility Of *An. gambiae* s.l. To Pirimiphos-methyl and Bendiocarb

An. gambiae s.l. populations were tested against bendiocarb and pirimiphos methyl in eight sites surveyed Susceptibility to pirimiphos methyl was recorded in all of the sites but for bendiocarb, *An. gambiae* s.l. populations of Koungheul was susceptible. Resistance suspected to bendiocarb was observed in Tambacounda and Vélingara, while the An. gambiae s.l. populations of Koumpentoum was resistant (Figure 26).

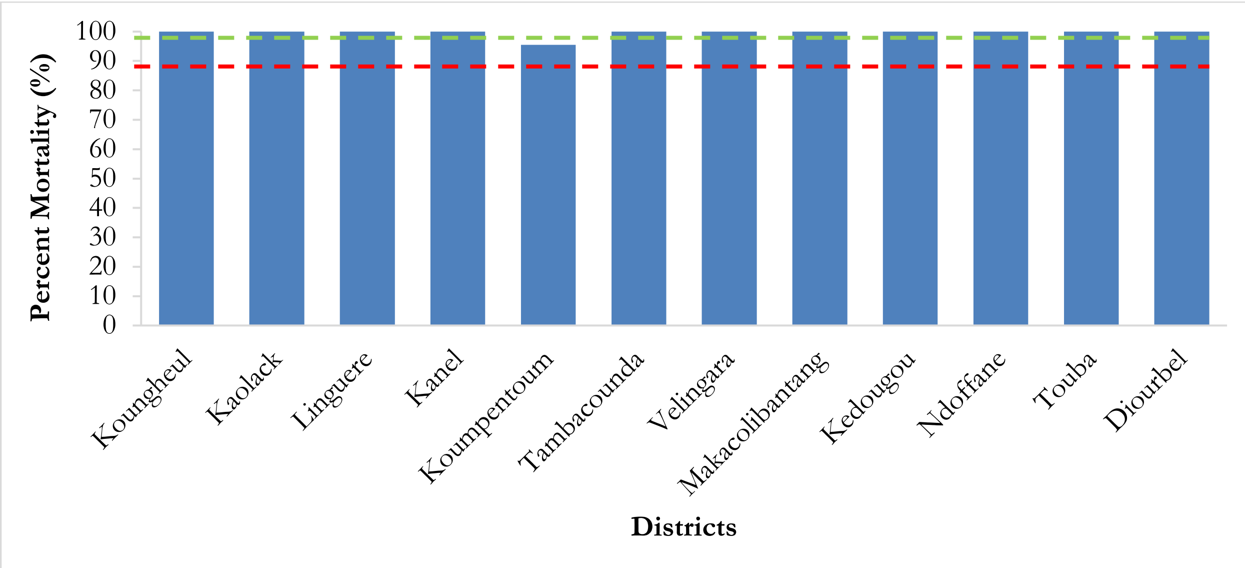
Figure 26: Susceptibility of *An. gambiae* s.l. to pirimiphos-methyl and bendiocarb



3.4.4 Susceptibility Of *An. gambiae* s.l. To Clothianidin

Susceptibility of *An. gambiae* s.l. to clothianidin 4 µg/bottle was recorded at all sites tested, with 100% mortality recorded from one day post exposure at all sites, except Koumpentoum (95.5%). The susceptibility test with clothianidin was done in 12 sites across the country (Figure 27).

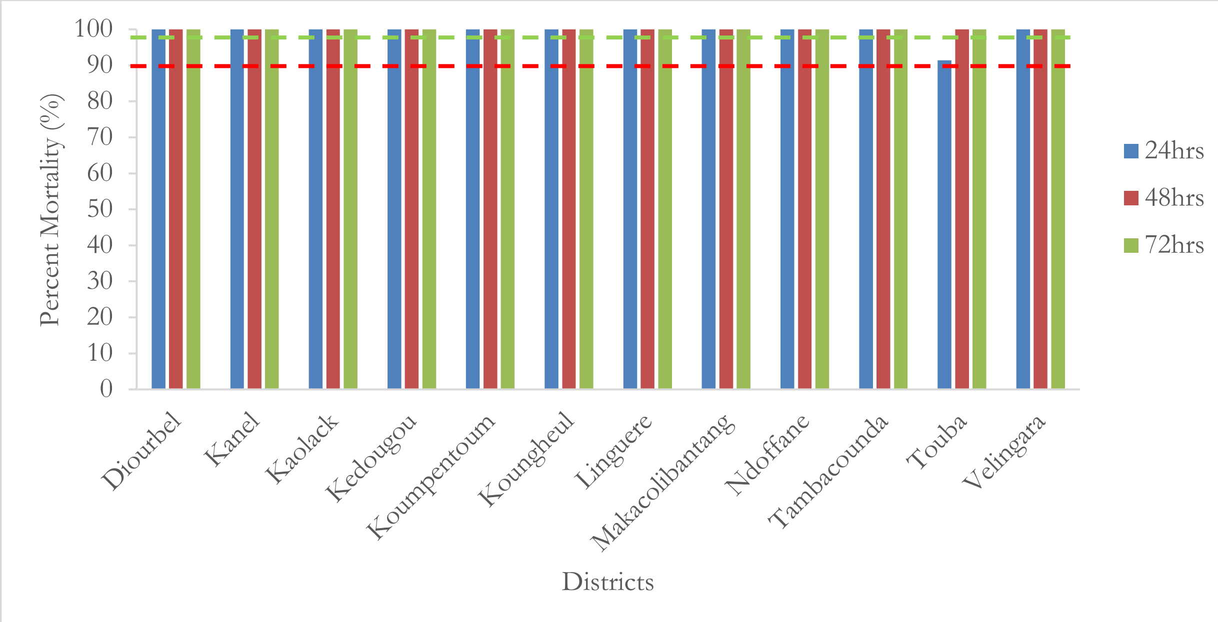
Figure 27: Susceptibility of *An. gambiae* s.l. to Clothianidin 4 µg/bottle



3.4.5 Susceptibility Of *An. gambiae* s.l. To Chlorfenapyr

One hundred percent mortality of *An. gambiae* s. l. was recorded against chlorfenapyr 100 µg/bottle at 11 out of 12 sites surveyed. It is only in Touba where, 91.4% of the mosquitoes were dead up to 24h (Figure 28).

Figure 28: Susceptibility of *An. gambiae* s.l. to Chlorfenapyr 100µg/Bottle



3.5 *AN. STEPHENSI* IN URBAN AREA OF DAKAR

Collection done at the end of the rainy season in October 2021 in about 29 areas around the Autonomous Port of Dakar did not yield any mosquitoes. The reservoirs on site were hermetically sealed, vases and others that can contain water are used for daily activities, which hinders mosquitoes from keeping their eggs for a long time. The only water point found was only positive for Culicinae larvae.

Positive larval habitats were recorded within the *Leopold Sedar SENGHOR* Airport and the surrounding areas compared to the port. The types of larval habitats were open containers, barrows and abandoned utensils with water. The collected larvae were reared to adults and identified. A total of 187 specimens were morphologically identified as *An. gambiae* s.l. and were stored in Eppendorf tubes with silica gel for further molecular identification by PCR. The PCR identification in 153 specimens showed that 132 (71%) were *An. arabiensis* and the 21 were negative for *An. gambiae* PCR. The lab plans for a second test of the 21 specimens before advising for *An. stephensi* sequencing at CDC Atlanta.

4. Discussion and Conclusion

4.1 Species Composition And Vector Density

Anopheles gambiae s.l. remains the predominant *Anopheles* species and predominant malaria vector collected using HLCs and PSCs at most surveillance sites in Senegal. *Anopheles* species diversity observed was similar to that observed in previous years, with the presence of *An. funestus* s.l., *An. pharoensis*, *An. rufipes*, *An. nili, An. squamosus* and *An. coustani*, *An. ziemanni* in addition to *An. gambiae* s.l. Of the two main malaria vectors in the region, *An. gambiae* s.l. remains dominant over *An. funestus* s.l., with an overall proportion of 78.9%. Furthermore, *An. funestus* s.l. was largely collected in Ndoffane, located in the Sudano-Sahelian zone of the country. It has previously been reported that the geographical location of these sites in Keur Momar sarr and in Ndoffane by a river is the main reason for the proliferation of *An. funestus* s.l.

Four species of the *An. gambiae* complex were observed, including *An. gambiae*, *An. arabiensis*, *An. coluzzii,* and *An. melas*. A few *An. gambiae*/*An. coluzzii* hybrids were also recorded. Interestingly, the vector population of most of the sites was primarily *An. arabiensis*. Only the Sudano-Guinean zone displayed a predominance of *An. gambiae* over the other members of the complex.

An. gambiae s.l. was also the predominant vector collected in all IRS districts and associated control sites. About 98% and 94% of the *Anopheles* population were *An. gambiae* s.l. in IRS and control sites respectively. Furthermore, *An. arabiensis* represented the main vector in the SumiShield-sprayed sites, while *An. gambiae* was mainly collected in Kédougou, where Fludora Fusion was sprayed. This trend remained similar to that of last year and reveals the stability of vector population diversity.

At both IRS and control sites, vector indoor densities and HBRs increased after IRS at most sites relative to in the period before IRS. These increases post-IRS may reflect the seasonal increases following the rainy season in the country that occurred between July and October. However, the timing of IRS also considers the fact for effective impact on vector density and malaria transmission. Vector densities and compositions were aggregated across site to compare sprayed versus control sites over the collection period. Any immediate and significant decrease in vector numbers was observed among sites and among site's status (sprayed or unsprayed) due to the rainy season timing of both the entomological data collection and IRS implementation.

4.2 Vector Biting Behavior

The overall mean HBR of *An. gambiae* s.l. was less than 10.0 b/p/n in all geographic zones with the highest(9.1 b/p/n) in the Sudano-Guinean and Sudano-Sahelian zones and the lowest (0.3 b/p/n) in the Sahelo-Sudanese zone. The low HBR in Sudanese zone could be explained by both implementations of IRS (Koumpentoum and Makacolibantang and the use of PBO nets in Tambacounda district. The mean endophagic rate showed a slightly higher outdoor biting by *An. gambiae* s.l. females overall even the vectors bite similarly indoors and outdoors in the Sudano-Guinean and Sudanese zones. Overall, the highest HBRs were recorded during the rainy season period (August to October) September within all geographical areas. The mean hourly peak biting occurred mostly during the second half of the night both indoors and outdoors. As in the previous years the mean HBR of *An. funestus* s.l. was very low except in Ndoffane with a peak of bites in July 2021, in outdoors. *An. funestus* s.l. remains exophagic most of the time with hourly biting peak between the second half of the night both outdoors (8:00 p.m-03:00 a.m.) and indoors (03:00 a.m-04:00 a.m.).

4.3 VECTOR INDOOR RESTING DENSITY STATUS, PARITY RATES, AND MALARIA TRANSMISSION INDICATORS

The highest IRDs were recorded in Sudano-Sahelian (2.7 f/r) zone, while the lowest IRDs were recorded in the Sudano-Guinean zone. This could explain the exophilic trends of the vector in the SudanoGuinean zone. The outdoor resting collections with prokopack aspiration yielded few Anopheles mosquitoes identified as An. gambiae s.l. This can be explained by the use of PBO nets in these sentinel sites which can impact on the reduction of the vectors. Furthermore, due to limited outdoor resting spaces, very few mosquitoes were collected. More investigations will need to be conducted to locate any hiding and resting places of the Anopheles vectors outdoors.

The mean *An. gambiae* s.l. parity rate of all sites surveyed was 57% over the collection period. But in the disaggregated IRS and control sites, the mean parity rate in the IRS sites was 61.5%, and that of the control sites was 54.1%, indicating that there is no difference between the sites. Parity rates are monitored to determine approximately the age structure of a vector population, as the older the vector population is, the higher is the likelihood of malaria transmission. They are also used to determine whether vector control efforts were successful, by reducing the overall age of the population The trends observed in the sprayed sites need to be closely considered, as the parity rate represents an important indicator within IRS entomological impact evaluation.

EIR of *An. gambiae* s.l. populations varied according to the geographical zones and was higher in the Sudanese and the Sudano-Guinean zones. Even though EIRs recorded were high enough to maintain the transmission of malaria in IRS districts located in Sudanese and Sudano-Guinean zones, they were significatively higher in their respective control sites suggesting that interventions may have contributed to the reduction in vector transmission. Infected *An. arabiensis*, *An. gambiae* and *An. coluzzii* females were found. The highest levels of infection recorded were obtained in *An. gambiae* in the Sudano-Guinean zone in Kedougou's IRS control sites. One female out of 18 of *An. funestus* tested was infected in Salemata.

An. stephensi was not identified morphologically in Dakar, but 187 *Anopheles* larvae identified as *An. gambiae* s.l. were collected from artificial containers near the Dakar airport. The PCR identification confirmed that 71% of 153 were *An. arabiensis*. The remaing speciemens 21 nagative for *Gambiae* PCR will be tested again before doing for them PCR for *Stephensi.*

4.4 REMANENCE OF SUMISHIELD AND FLUDORA FUSION

Both SumiShield and Fludora Fusion were effective on both mud and cement walls for at least eight months. Both insecticides were used for the second time in Senegal and the residual efficacy recorded is encouraging for further spray campaigns and the insecticide rotation strategy. Despite this effectiveness with susceptible strain of *An. coluzzii*, it is also important to see the effect of these new products on local wild populations of *An. gambiae* s.l.

4.5 INSECTICIDE SUSCEPTIBILITY

Pyrethroid resistance intensity testing was done in 7 of the 16 targeted sites with moderate resistant observed to all three pyrethroids except in Velingara (Sudano Guinean) for deltamethrin and in Tambacounda (Sudanese) for alpha-cypermethrin where high resistant was recorded. Fortunately, pre-exposure to piperonyl butoxide increased substantially the mortality of the vectors in most of the sites tested and particularly for deltamethrin and permethrin in most of the cases including KKT zone where the NMCP plans to distribute PBO nets during the 2022 mass campaign.

Susceptibility recorded against clothianidin and pirimiphos methyl is of interest for IRS insecticide decision-making as NMCP plans to use Fludora Fusion, SumiShield and Actellic in IRS 2022. Susceptibility against chlorfenapyr also is of interest for dual insecticide- ITN procurements.

4.6 CONCLUSION

The data gathered during the 2021 entomological activities will continue to support the NMCP in malaria vector control decision-making with regards to IRS campaigns and ITN distributions in the country. The activities in the surveyed sites will help better characterize and understand vector behavior for appropriate vector control implementation.

Overall, the vector populations of all surveyed sites were found biting slightly more outdoors than indoors. Similar trends have been already described within the previous year's reports. Outdoor biting has always been a cause for concern in the country, as all vector control strategies currently focus on indoor biting and resting behaviors. The vectors are mostly *An. arabiensis*, which could explain the trend, as the species is mostly reported to be more exophilic among the species of the complex, though there are some cases of heterogeneity behavior.

The peak biting of *An. funestus* s.l. at the beginning of the rainy season could be targeted for future evaluation of *An. funestus* s.l. regarding the susceptibility status against the insecticides used for vector control (IRS and ITNs).

4.7 CHALLENGES

The 2021 insecticide resistance monitoring encountered a lot of challenges, namely: the late delivery of insecticide impregnated papers, causing the missing of the rainy season for the collection of enough larvae for tests, some of the selected sites to be surveyed dried before the delivery of the impregnated papers.

The laboratory data collection could not be completed due to delay in reagent delivery and particularly the ELISA of blood meal sources. Additionally, the delay in reagent delivery is still slowing the laboratory activities not enabling the PCR work for the genotyping of the insecticide resistance markers among the mosquitoes tested for susceptibility.

The large number of sites to be surveyed is still a big challenge for easy data collection at all the sites with a limited number of field technicians. Furthermore, some of the sites were actively monitored for the past five years and the trends have not changed drastically.

5. RECOMMENDATIONS

- Outdoor biting was observed at many sites. Larval source management (LSM) could be considered as a complementary vector control intervention which targets outdoor biting mosquitoes to help reduce population densities in sites like Koungheul or Tambacounda in addition to the ongoing interventions. Secondly, urban sites like Kedougou could be monitored for larval habitats in addition to the previously assessed sites (Touba, Kaolack and Diourbel) for LSM consideration. A first step would be to identify areas where it would be feasible to conduct these studies.
- Given that Senegal is using the clothianidin-based insecticide for the second time for IRS, rotating insecticide is necessary, and another class (organophosphate: pirimiphos methyl) will be added in 2022. The same previous insecticides could be considered for the 2022 IRS but rotated following PMI insecticide procurement guidance.
- Due to the continued resistance of local vectors to pyrethroid insecticides in some areas, consider expanding the use of piperonyl butoxide nets or introducing dual active ingredient nets, especially where ITNs are the only vector control intervention. Given the susceptibility to chlorfenapyr observed at all sites a dual active ingredient net such as Interceptor G2 may be appropriate either nationwide or in high pyrethroid resistance areas.
- Reduce the number of longitudinal monitoring entomological sentinel sites and focus on sites where intense vector control strategies are implemented. Maintain other sites for insecticide resistance monitoring only.

Annex A: Anopheles species composition and behavior

Table A1: Species Composition by Geographical Zone

Geographical area	*An. gambiae s.l.*	*An. funestus s.l.*	*An. rufipes*	*An. pharoensis*	*An. nili*	*An. coustani*	*An. ziemanni*	*An. squamosus*	*Total*
Sahelian	79 (16.2)	403 (82.7)	3 (0.6)	1 (0.2)	0.0%	0.0%	1 (0.2)	0.0%	487
Sahelo-Sudanese	450 (87.9)	1 (0.2)	50 (9.8)	11 (2.2)	0.0%	0.0%	0.0%	0.0%	512
Sudano-Sahelian	3960 (65.0)	2063 (33.9)	48 (0.8)	16 (0.3)	0.0%	0.0%	5 (0.1)	0.0%	6092
Sudanese Zone	2063 (95.8)	1 (0.1)	78 (3.6)	11 (0.5)	0.0%	0.0%	0.0%	0.0%	2153
Sudano-Guinean	4517 (94.5)	38 (0.8)	45 (0.94)	19 (0.4)	140 (2.9)	19 (0.4)	0.0%	1 (0.02)	4779
Total	**11069 (78.9)**	**2506 (17.9)**	**224 (1.6)**	**58 (0.4)**	**1.0%**	**0.1%**	**6 (0.04)**	**1 (0.01)**	**14023**

Table A2: Human Biting Rate and Endophagic Rate by Site and Geographical Zone

Geographical zone	HLC	Number human-night	HBR	Indoor	Outdoor	Endophagic rate
Sahelo-Sudanese Zone	87	336	0.26	65	22	0.75
Sudano-Sahelian Zone	2131	576	3.70	963	1168	0.45
Sudanese Zone	814	1006	0.81	392	422	0.48
Sudano-Guinean Zone	4139	454	9.12	2070	2069	0.50
Total	**7171**	**2372**	**3.02**	**3490**	**3681**	**0.49**

Table A3: Indoor Resting Densities by Geographical Zone

Geographical zone	*An. gambiae* s.l.	Rooms	IRD
Sahelian Zone	79	140	0.6
Sahelo-Sudanese Zone	363	300	1.2
Sudano-Sahelian Zone	1829	680	2.7
Sudanese Zone	1249	1110	1.1
Sudano-Guinean Zone	378	599	0.6
Country	3898	2829	1.4

Table A4: Species Composition in IRS districts and controls

Statut	District	*An. gambiae s.l.*	*An. funestus s.l.*	*An. rufipes*	*An. pharoe nsis*	*An. nili*	*An. coustani*	*An. ziem anni*	*Total*
IRS	Koumpentoum (IRS)	230	0	3	2	0	0	0	235
	Maka Colibantang (IRS)	221	0	20	0	0	0	0	241
Control	Tambacounda (Control)	1612	1	55	9	0	0	0	1677
IRS	District Kedougou	1984	3	2	5	9	0	0	2003
Control	District Salemata	1359	22	11	11	122	18	0	1543
	District Saraya	1023	11	0	3	9	1	0	1047
IRS	District Koungheul	1397	2	18	11	0	0	5	1433
Control	District Malem Hodar	100	0	0	0	0	0	0	100
Total IRS		**3832**	**5**	**43**	**18**	**9**	**0**	**5**	**3912**
Total Control		**4094**	**34**	**66**	**23**	**131**	**19**	**0**	**4367**
Total		**7926**	**39**	**109**	**41**	**140**	**19**	**5**	**8279**
% IRS et Control		95.70%	0.50%	1.30%	0.50%	1.70%	0.20%	0.10%	
IRS districts		98.00%	0.10%	1.10%	0.50%	0.20%	0.00%	0.10%	
Controls districts		93.70%	0.80%	1.50%	0.50%	3.00%	0.40%	0.00%	

Table A5: Human Biting Rate in IRS districts and controls

<table>
<tr><th>District</th><th></th><th>An. gambiae s.l.</th><th>H/N</th><th colspan="2">HBR</th></tr>
<tr><td rowspan="2">Koungheul</td><td>Indoor</td><td>156</td><td>96</td><td>1.6</td><td rowspan="2">1.8</td></tr>
<tr><td>Outdoor</td><td>196</td><td>96</td><td>2.0</td></tr>
<tr><td rowspan="2">Koumpentoum</td><td>Indoor</td><td>35</td><td>96</td><td>0.4</td><td rowspan="2">0.4</td></tr>
<tr><td>Outdoor</td><td>47</td><td>96</td><td>0.5</td></tr>
<tr><td rowspan="2">Makacolibantang</td><td>Indoor</td><td>24</td><td>84</td><td>0.3</td><td rowspan="2">0.4</td></tr>
<tr><td>Outdoor</td><td>36</td><td>84</td><td>0.4</td></tr>
<tr><td rowspan="2">Kedougou</td><td>Indoor</td><td>851</td><td>96</td><td>8.9</td><td rowspan="2">9.8</td></tr>
<tr><td>Outdoor</td><td>1033</td><td>96</td><td>10.8</td></tr>
<tr><td rowspan="2">Malem Hodar</td><td>Indoor</td><td>6</td><td>84</td><td>0.1</td><td rowspan="2">0.1</td></tr>
<tr><td>Outdoor</td><td>7</td><td>84</td><td>0.1</td></tr>
<tr><td rowspan="2">Tambacounda</td><td>Indoor</td><td>335</td><td>276</td><td>1.2</td><td rowspan="2">1.2</td></tr>
<tr><td>Outdoor</td><td>338</td><td>276</td><td>1.2</td></tr>
<tr><td rowspan="2">Salemata</td><td>Indoor</td><td>692</td><td>48</td><td>14.4</td><td rowspan="2">13.5</td></tr>
<tr><td>Outdoor</td><td>604</td><td>48</td><td>12.6</td></tr>
<tr><td>Saraya</td><td>Indoor</td><td>518</td><td>48</td><td>10.8</td><td>9.8</td></tr>
</table>

	Outdoor	425	48	8.9	
	Total IRS + control	**5303**	**1656**	**3.2**	
IRS FF (Kedougou)	Indoor	36	84	0.4	4.9
	Outdoor	851	96	8.9	
	Total FF	**887**	**180**	**4.9**	
Control FF (Saraya and Salemata)	Indoor	1211	96	12.6	11.7
	Outdoor	1028	96	10.7	
	Total Control	**2239**	**192**	**11.7**	
IRS SS (Koungheul, Koumpentoum and Makacolibantang)	Indoor	215	276	0.8	0.9
	Outdoor	278	276	1.0	
	Total SS	**493**	**552**	**0.9**	
Control SS (Malem Hodar and Tambacounda)	Indoor	341	360	0.9	1.0
	Outdoor	346	360	1.0	
	Total Control	**686**	**720**	**1.0**	

Table A6: Indoor Resting Densities in IRS districts and controls

District	***An. gambiae s.l.***	**Rooms**	**IRD**
Koumpentoum	149	190	0.8
Makacolibantang	161	180	0.9
Tambacounda	939	540	1.7
Kedougou	94	200	0.5
Salemata	57	100	0.6
Saraya	77	100	0.8
Koungheul	1043	200	5.2
Malem Hodar	89	180	0.5
IRS districts	1447	770	1.9
Controls districts	1162	920	1.3
Fludora districts	94	200	0.5
Fludora controls	134	200	0.6
SumiShield districts	1353	570	0.8
SumiShield controls	1028	720	5.2
IRS and controls	2609	1690	0.5

Annex B: laboratory, cone bioassay and susceptibility test data

Table B1: *An. gambiae* s.l. and *An. funestus* composition in the geographical zone (January to December 2021)

Geographical zone	*An. gambiae* s.l. (%)					*An. funestus* (%)	
	An. arabiensis	*An. coluzzii*	*An. gambiae*	*Hybrid An. gambiae/An. coluzzii*	*An. melas*	*An. funestus ss*	*An. rivulorum*
Sahelian	64 (100)	-	-	-	-	98 (89.91)	11 (10.09)
Sahelo-Sudanese	225 (98.68)	3 (1.32)	-	-	-	1 (100)	-
Sudano-Sahelian	1383 (88.43)	150 (9.59)	16 (1.02)	-	15 (0.96)	483 (92.71)	38 (7.29)
Sudanese	547 (53.26)	72 (7.01)	404 (39.34)	4 (0.39)	-	-	-
Sudano-Guinean	344 (16.66)	61 (2.95)	1655 (80.15)	5 (0.24)	-	6 (75)	2 (25)

Table B2: *An. gambiae* s.l. species composition in the surveyed districts (January to December 2021)

Geographical zone	*An. gambiae* s.l. (%)						*An. funestus* (%)		
	Total	*An. arabiensis*	*An. gambiae*	*An. coluzzii*	*Hybrid An. gambiae/An. coluzzii*	*An. melas*	Total	*An. funestus ss*	*An. rivulorum*
Sahelian									
Richard Toll	64	64 (100)	-	-	-	-	109	98 (89.9)	11 (10.1)
Sahelo-Sudanese									
Kanel	61	59 (96.7)	-	2 (3.3)	-	-	0		-
Linguere	167	166 (99.4)	-	1 (0.6)	-	-	1	1 (100.0)	-
Sudano-Sahelian									
Diourbel	39	39 (100.0)	-	-	-	-	0	-	-
Kaolack	520	520 (100.0)	-	-	-	-	0	-	-
Koungheul	640	492 (76.9)	11 (1.7)	137 (21.4)	-	-	0	-	-
Malem hodar	73	68 (93.2)	2 (2.7)	3 (4.1)	-	-	0	-	-
Ndoffane	248	224 (90.3)	1 (0.4)	8 (3.2)	-	15 (6.1)	521	483 (92.7)	38 (7.3)
Touba	40	40 (100.0)	-	-	-	-	0	-	-
Sudanese									
Koumpentoum	143	115 (80.4)	11 (7.7)	16 (11.2)	1 (0.7)	-	0	-	-
Makacolibantang	137	91 (66.4)	33 (24.1)	12 (8.8)	1 (0.7)	-	0	-	-
Tambacounda	345	241 (69.8)	78 (22.6)	25 (7.3)	1 (0.3)	-	0	-	-
Tambacounda PBO	402	100 (24.9)	282 (70.2)	19 (4.7)	1 (0.3)	-	0	-	-
Sudano-Guinean									
Kedougou	921	156 (16.9)	741 (80.5)	23 (2.5)	1 (0.10)	-	1	1 (100.0)	-
Salemata	600	90 (15)	500 (83.3)	8 (1.3)	2 (0.3)	-	4	4 (100.0)	-
Saraya	428	36 (8.4)	373 (87.2)	17 (4.0)	2 (0.5)	-	3	1 (33.3)	2 (66.7)
Velingara	116	62 (53.5)	41 (35.3)	13 (11.2)	-	-	0	-	-

Table B3: Monthly Frequencies of *An. gambiae* s.l. species collected by HLC and PSC from the geographical zones surveyed (January to December 2021)

Species	Geographical zone	January	February	May	June	July	August	September	October	November	December
An. arabiensis											
	Sahelian			25	2.32	0.4	1.96	8.02	0.81	0.45	
	Sahelo-Sudanese			12.5	1.55	1.21	3.93	5.88	18.36	18.46	
	Sudano-Sahelian	29.41			56.58	60.72	81.74	59.89	42.85	51.35	28.9
	Sudanese	11.76		12.5	3.87	16.19	10.95	25.31	27.14	24.09	25.08
	Sudano-Guinean	58.82		50	35.65	21.45	1.4	0.89	10.81	5.63	45.98
An. coluzzii											
	Sahelo-Sudanese							0.82	3.12		
	Sudano-Sahelian				100		64.44	57.02	65.62	40	6.25
	Sudanese					15.78	24.44	29.75	25	25	6.25
	Sudano-Guinean					84.21	11.11	12.39	6.25	35	87.5
An. gambiae											
	Sahelian										
	Sahelo-Sudanese										
	Sudano-Sahelian						0.85	1.13	1.67		
	Sudanese					0.32	24.06	36.81	14.48	3.52	
	Sudano-Guinean			100	100	99.67	75.07	62.03	83.84	96.47	100
Hybrid *An. gambiae/An. coluzzii*											
	Sudanese					100	100	33			
	Sudano-Guinean							66.66	100		
An. melas											
	Sudano-Sahelian				100	100					

Table B4: Monthly Infection Rate of *An. gambiae* s.l. (January to December 2021)

Districts	January		February		May		June		July		August		September		October		November		December	
	T	P	T	P	T	P	T	P	T	P	T	P	T	P	T	P	T	P	T	P
Richard Toll	-	-	-	-	3	0	3		3	0	8	0	51	0	4	0	3	0	-	-
Kanel	-	-	-	-	-	-	-	-	-	-	6	0	3	0	12	0	2	0	-	-
Linguere	-	-	-	-	-	-	-	-	-	-	1	0	3	0	19	0	10	0	-	-
Diourbel	-	-	-	-	-	-	-	-	-	-	12	0	12	0	1	0	7	0	8	0
Touba	-	-	-	-	-	-	-	-	10	0	5	0	-	-	8	1	63	0	4	0
Kaolack	-	-	-	-	-	-	8	0	98	0	166	0	156	0	32	0	36	0	10	0
Koungheul	4	0	-	-	-	-	1	0	5	0	23	0	100	0	75	0	114	0	33	0
Malem hodar	1	0	-	-	-	-	-	-	2	0	3	0	4	0	1	0	-		-	-
Ndoffane	-	-	-	-	-	-	28	0	10		4	0	19	0	9	0	3	0	7	
Koumpentoum	-	-	-	-	1	0	-	-	-	-	4	0	17	1	5	0	4	0	-	-
Makacolibanta	-	-	-	-	-	-	-	-	4	0	8	0	15	0	21	0	1	1	-	-
Tamba PBO	1	0	-	-	-	-	-	-	3	0	155	0	99	1	11	0	16	0	9	0
Tambacounda	-	-	-	-	-	-	-	-	3	0	16	0	63	0	96	2	29	1	7	0
Salemata	7	0	-	-	1	0	112	0	94	2	86	5	78	1	86	1	52	2	64	0
Saraya	5	0	-	-	-	-	4	0	99	0	77	3	96	2	70	3	1	0	21	0
Kedougou	8	0	-	-	-	-	7	0	150	2	198	4	159	0	153	1	138	5	44	0
Velingara	-	-	-	-	2	0	3	0	11	0	9	0	19	1	46	0	18	0	36	0

T=tested, P = positive

Table B5: Monthly Infection Rate of *An. funestus* (January to December 2021)

Districts	January		February		May		June		July		August		September		October		November		December	
	T	P	T	P	T	P	T	P	T	P	T	P	T	P	T	P	T	P	T	P
Kedougou	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	0	-	-	-	-
Koungheul	-	-	-	-	-	-	-	-	-	-	-	-	1	0	-	-	-	-	-	-
Ndoffane	-	-	-	-	-	-	105	0	85	0	33	0	78	0	46	0	81	0	91	0
Richard Toll	-	-	-	-	13	0	16	0	3	0	21	1	77	0	137	1	95	0	-	-
Salemata	-	-	-	-	-	-	-	-	-	-	-	-	5	0	4	0	1	0	1	0
Saraya	-	-	-	-	-	-	-	-	-	-	3	0	1	1	4	0	-	-	-	-
Velingara	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	0	-	-

T=tested, P = positive

Table B6: Infection Rate of *An. pharoensis* and *An. nili* by Geographic Zone (January to December 2021)

Geographical areas	Districts	*An. pharoensis*			*An. nili*		
		T	**P**	**CSI**	**T**	**P**	**CSI**
Sahelian	Richard Toll	1	0	0	-	-	-
Sahelo-Sudanese	Kanel	5	0	0	-	-	-
	Linguere	4	0	0	-	-	-
Sudano-Sahelian	Touba						
	Diourbel						
	Kaolack	4	0	0	-	-	-
	Koungheul	11	0	0	-	-	-
	Malem_hodar				-	-	-
	Ndoffane	2	0	0	1	0	0
Sudanese	Koumpentoum	1	0	0			
	Makacolibantang						
	Tambacounda	3	0	0			
	Tambacounda (PBO-LLINs-site)*	6	0	0			
Sudano-Guinean	Kedougou	5	0	0	9	0	0
	Salemata	10	0	0	67	0	0
	Saraya	3	0	0	9	0	0
	Vélingara	0	0	0			

T = Tested ; P= Posittive ; CSI = Circumsporozoite index

Table B7: *Plasmodium Falciparum* Infection Rate of *An. gambiae* s.l. Species in the Surveyed Sites (january to december 2021)

Districts	*An. arabiensis*					*An. gambiae*				*An. coluzzii*				*Hybrid An. gambiae/coluzzii*		
	T		**P**	**CSI**		**T**	**P**	**CSI**		**T**	**P**	**CSI**		**T**	**P**	**CSI**
Richard Toll*	63		0													
Kanel	22		0													
Linguere	30		0													
Touba*	41		1	0.024												
Diourbel*	31		0													
Kaolack	481		0													
Tambacounda (PBO-LLINs-site)*	43		0			157	1	0.006		5	0	0		1	0	0
Ndoffane	67		0							1	0	0				
Velingara*	62		0	0		41	0	0		13	1	0.077				

T = Tested ; P= Posittive ; CSI = Circumsporozoite index

Table B8: Indoor and Outdoor Entomological Inoculation Rate of *An. gambiae* s.l. Females in the Surveyed Sites (January to December 2021)

Geographic zone	District	Indoor			Outdoor			Total		
		HBR	CSI	EIR	HBR	CSI	EIR	HBR	CSI	EIR
Sahelian	Richard Toll*	-	-	-	-	-	-	-	-	-
Sahelo-sudanese	Kanel	0.12	0		0.11	0		0.12	0	
	Linguere	0.21	0		0.14	0		0.17	0	
Sudano-sahelian	Touba*	-	-	-	-		-		0.011	
	Diourbel*	-		-	-		-			
	Kaolack	17.95	0		21.33	0		19.64	0	
	Koungheul (IRS)	1.44	0		1.85	0		1.64	0	
	Malem_hodar (Control)	0.05	0		0.06	0		0.051	-	
	Ndoffane	1.07	0		1.5	0				
Sudanese	Koumpentoum (IRS)	0.16	0.059	0.009	0.13			0.14	0.033	0.005
	Makacolibantang (IRS)	0.25	0		0.38	0.031	0.012	0.31	0.021	0.006
	Tambacounda (Control)	1.32	0		1.45	0.025	0.036	2.73	0.014	0.038
	Tambaounda PBO	0.95	0		0.88	0.007	0.006	0.91	0.003	0.003
Sudano-Guinean	Kedougou (IRS)	7.88	0.009	0.071	9.62	0.018	0.17	8.75	0.014	0.122
	Velingara*	-		-	-		-		0.007	
	Salemata (Control)	12.98	0.027	0.350	11.24	0.01	0.112	12.11	0.019	0.230
	Saraya (Control)	9.83	0.025	0.245	7.61	0.02	0.152	8.72	0.021	0.183

*Mosquitoes Collected by PSC only; HBR = human-biting rate ; CSI = Circumsporozoite index ; EIR = entomological inoculation rate

ANNEX C: SUSCEPTIBILITY TEST DATA

Table C1. Insecticide Susceptibility Testing Activities by District

Geographical zone	Districts	Chlorfenapyr	Clothianidin	Deltamethrin			Permethrin			Alpha-cypermethrin			PBO+PY	Bendiocarb	Pirimiphos - methyl
		100 µg/ml	4 µg/ml	1X	5X	10X	1X	5X	10X	1X	5X	10X			
	Richard Toll														
	Linguère												Alpha		
SAHELIAN	Kanel												Alpha		
	Diourbel														
	Kaolack												Delta		
	Touba												Delta/Perm		
	Malem Hodar														
	Ndoffane														
SUDANO SAHELIAN	Koungheul												Delta/Perm/Alpha		
	Koumpentoum												Delta/Perm/Alpha		
	Makacolibantang														
SUDANESE	Tamba												Delta/Perm		
	Kédougou												Delta/Perm		
	Saréya														
	Salémata														
SUDANO GUINEAN	Vélingara												Delta		

Tests performed

Tests not performed

ANNEX D: BIBLIOGRAPHY

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