PMI VECTORLINK PROJECT

THE SENEGAL

ANNUAL ENTOMOLOGICAL REPORT

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ACRONYMS

AR	anthropophilic rate
b/p/n	bite per person per night
CBS	community-based surveillance
CDC	U.S. 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
NMCP	National Malaria Control Program
ORC	outdoor resting collection
PBO	piperonyl butoxide
PCR	polymerase chain reaction
PMI	U.S. President's Malaria Initiative
WHO	World Health Organization
w/w	weight for weight

EXECUTIVE SUMMARY

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

In 2022, VectorLink Senegal supported the National Malaria Control Program (NMCP) to implement IRS in four districts: Kédougou, Makacolibantang, Koumpentoum, and Koungheul. A single spray campaign was conducted in all districts from May 30 through June 24, 2022, using two clothianidin-based formulations (SumiShield in Kédougou and Fludora Fusion in Koumpentoum and Koungheul) and Actellic in Makacolibantang. During the IRS campaign, spray operators enumerated a total of 142,347 structures, of which they sprayed 138,752, for a spray coverage rate of 97.5%. Additionally, PMI procured 1,075,774 ITNs for the project, of which 1,018,974 ITNs were distributed to 14 health regions.

To assess the effectiveness and impact of these vector control interventions, PMI VectorLink Senegal and its subcontractor, the Laboratory of Vector and Parasite Ecology (Laboratoire d'Ecologie Vectorielle et Parasitaire) of Cheikh Anta Diop University, conducted entomological monitoring activities in selected sentinel sites across the country. The sites were selected together with the NMCP per their priorities to assess transmission trends in the different geographical zones within the country in addition to the monitoring of the IRS and control sites, and PBO-ITN sites for estimating the entomological impact of the interventions. Longitudinal vector surveillance and insecticide resistance monitoring were conducted in 27 sentinel sites spread across five geographical zones (Sahelian, Sahelo-Sudanese, Sudanese, Sudano-Sahelian, and Sudano-Guinean) within the 16 health districts of the country from April to December 2022. Adult mosquito collections were conducted monthly using human landing catches (HLC), pyrethrum spray catches (PSC), and U.S. Centers for Disease Control and Prevention light traps (CDC-LT). Furthermore, outdoor resting collections (ORC) using Prokopack aspiration were conducted monthly in two zones (Sudanese and Sudano-Guinean) to investigate the resting behavior of the different *Anopheles* species collected. Subsamples of preserved *An. gambiae* s.l. and *An. funestus* s.l. were screened for the presence of *Plasmodium falciparum* infection, for blood feeding source, and for species identification using enzyme-linked immunosorbent assay (ELISA) and polymerase chain reaction (PCR) assays, 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 susceptible mosquitoes dropped 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 September and November 2022 using the WHO tube test for adult mosquitoes and the WHO 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%), pirimiphos-methyl 0.25%, and bendiocarb 0.1% were determined using WHO test kits. Chlorfenapyr 100 µg/bottle and clothianidin 4 µg/bottle were tested using WHO bottle assays.

A total of 5,675 *Anopheles* mosquitoes of 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 through human landing catches, pyrethrum spray catches, and Centers for Disease Control and Prevention light traps. *An. gambiae* s.l. was predominant (86.1%), followed by *An. rufipes* (4.69%) and *An. nili* (3.72%). *An. squamosus* (0.04%) and *An. nili* (3.71%) were found only in the Sudano-Guinean zone, where all eight species were collected. *An. funestus* s.l. was mainly collected in the Sahelian zone, which is close to a river with vegetation.

Only 35 *Anopheles* mosquitoes were collected through outdoor resting collections., though the level of collection efforts was not the same as other collection methods.

An. arabiensis was the main species of the *An. gambiae* complex collected in the country. *An. gambiae* was mostly observed during the rainy season, especially in the Sudano-Guinean zone. *An. coluzzii* was recorded only during the rainy season in all zones except Sahelian. *An. funestus* s.s. was the sole species of the *An. funestus* group identified among the samples collected in the Sudano-Sahelian, Sudanese, and Sudano-Guinean zones.

The human biting rates were high only in the Sudano-Guinean zone, with more 20 bites per person per night (b/p/n). In other zones, rates were less than 1 b/p/n. *An. gambiae* s.l. was exophagic in Sudanese and Sudano-Guinean zones. Overall, *An. gambiae* s.l. human biting rates were higher in the second half of the night indoors than outdoors. At the IRS sites, *An. gambiae* s.l. bit more indoors than outdoors. In control sites, outdoor biting rates were higher than indoor.

The Sudano-Guinean zone recorded the lowest indoor resting density of *An. gambiae* s.l. (0.42 females per room) and the highest proportion of blood-fed females (60.0%). Only 35 *Anopheles* were collected outdoors with Prokopack of three species (85.7% *An. gambiae* s.l., 8.6% *An. funestus*, and 5.7% *An. rufipes*).

Average parity rates in IRS sites (52%) and in control sites (60%) showed slight decreases, but rates were not significantly different in sprayed districts, indicating that the vectors were old enough to transmit the parasites.

The highest anthropophilic rate was recorded in the Sudano-Guinean zone, where the majority of *An. gambiae* was collected, with a mean rate of 80.7%. In the other geographical zones where *An. arabiensis* was predominant, the anthropophilic rate was low, especially in the Sahelian zone, where it was less than 50%. *An. gambiae* s.s., An. *coluzzii* and hybrid of both species were the only vectors positives for *P. falciparum* sporozoites, which were detected in the Sudanese and Sudano-Guinean zones only and where malaria incidences are higher in country. Within the complex, no infected *An. arabiensis* or *An. melas* was detected. The hybrid *An. coluzzii*/*An. gambiae* recorded the highest *P. falciparum* rate (5.0%), followed by *An. gambiae* (2.0%) and *An. coluzzii* (1.0%). In the four IRS districts, infected females were found only in Kédougou (1.13%). Moreover, the sporozoite rate was significantly lower than in its control (Saraya/Salemata: 2.58%). No infected vector was also recorded in the other IRS districts (Koumpentoum or Makacolibantang) contrary to their control site of Tambacounda district (Lycounda: 0.49%).

Larval habitats that could be suitable for *An. stephensi* were found in Dakar around the seaport and airport and in Touba. From the collected larvae, emerged adults were morphologically identified as *An. gambiae* s.l. Molecular species identification of the samples confirmed 98% of the specimens as *An. arabiensis*. The remaining specimens (17) negative for *An. gambiae* complex species will be retested using *An. stephensi* primers.

From July 2022 to February 2023, wall cone bioassays were conducted on both mud and cement walls in all IRS districts using a laboratory-reared susceptible *An. coluzzii* strain, which showed the residual efficacy of all sprayed insecticides (Fludora Fusion, SumiShield, and Actellic) at above 80% of the efficacy threshold. The

results confirm that both clothianidin-based insecticides and Actellic were effective for at least eight months post spraying.

Pyrethroid (deltamethrin 0.05%, permethrin 0.75%, and alpha-cypermethrin 0.05%) resistance was observed in all sites and for all insecticides, except deltamethrin in Koungheul, where resistance was suspected (91%). PBO+deltamethrin reversed the resistance status of the *An. gambiae* s.l. to susceptibility in Koungheul, Koumpentoum, Makacolibantang, and Tambacounda. A partial increment of mortality was recorded in Keur Massar, Touba, Velingara, and Kédougou. PBO+permethrin tested in seven of the districts also reversed the resistance status of the *An. gambiae* s.l. only in Koungheul and Koumpentoum and partially increased mortality in Touba and Kédougou. With alpha-cypermethrin tested in nine districts, pre-exposure to PBO reversed the resistance status of the *An. gambiae* s.l. in Koungheul and Koumpentoum and increased partial mortality in Keur Massar, Touba, Tambacounda, Makacolibantang, and Kédougou. The resistance observed was either of moderate or low intensity at all tested sites, except with alpha-cypermethrin in Malem Hodar and permethrin in Tambacounda, where high resistance intensity was recorded. Furthermore, susceptibility to bendiocarb, pirimiphos-methyl, clothianidin, and chlorfenapyr was observed at all geographic zones.

Overall, the vector populations of all surveyed sites were reported biting slightly but not significantly more outdoors than indoors, with *An. arabiensis* being the main vector in all geographical zones. Similar trends have been described in previous years' reports. Outdoor biting remains a concern in the country, because of vector bionomics trends which report outdoor biting. Pyrethroid resistance was observed, however, other insecticide classes are still showing susceptibility against the vectors, and that represents an important parameter for vector control strategy and insecticide selection decision-making.

1. INTRODUCTION

In Senegal, malaria remains endemic and represents a major cause of morbidity and mortality, particularly among children under age 5 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 made substantial efforts in 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 National Malaria Control Program (NMCP) has received support from the U.S. President's Malaria Initiative (PMI) for indoor residual spraying (IRS), insecticide-treated net (ITN) distributions, and entomological monitoring since 2007. In 2021 and under the PMI VectorLink Project, IRS was conducted in Kédougou using Fludora Fusion and in Koungheul, Koumpentoum, and Makacolibantang using SumiShield. In 2022, IRS was conducted from May 30 to June 24 in the same districts, with Fludora Fusion in Koungheul and Koumpentoum, Actellic in Makacolibantang, and SumiShield in Kédougou. Furthermore, PMI provided the project with 1,075,774 ITNs stocked in the VectorLink's central warehouse, of which the project distributed 1,018,974 ITNs to 14 health regions.

In 2022, 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.

The data collected will be compiled by malaria vector control stakeholders (including PMI VectorLink) to inform the best timing of IRS campaigns as well as the selection and distribution of ITNs in alignment with the national insecticide resistant management strategy. Taken together this information will be used for decision-making by the NMCP.

2. METHODS

2.1 ENTOMOLOGICAL MONITORING SITES

Entomological surveillance started in April 2022 and was conducted in 27 sites, across 14 districts in five geographical zones (Figure 1). Sites included eight IRS sites (two per sprayed district), six IRS control sites (two per control district in Kédougou and Koungheul and one for Makacolibantang and Koumpentoum), four piperonyl butoxide (PBO) ITN distribution sites and six community-based surveillance (CBS) sites selected in five different districts. In addition, three sites (one in Touba and two in Dakar) were selected as *Anopheles stephensi* monitoring sites (Table 1). Districts were pooled by geographical zones for data analysis: (1) the Sahelian zone (Richard Toll and Keur Momar Sarr), (2) the Sahelo-Sudanese zone (Dakar), (3) the Sudano-Sahelian zone (Diourbel, Touba Malem Hodar, and Koungheul), (4) the Sudanese zone (Koumpentoum, Makacolibantang, and Tambacounda), and (5) the Sudano-Guinean zone (Kédougou, Salemata, Saraya, and Velingara) (Table 1). Furthermore, IRS sites were compared with associated control sites.

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

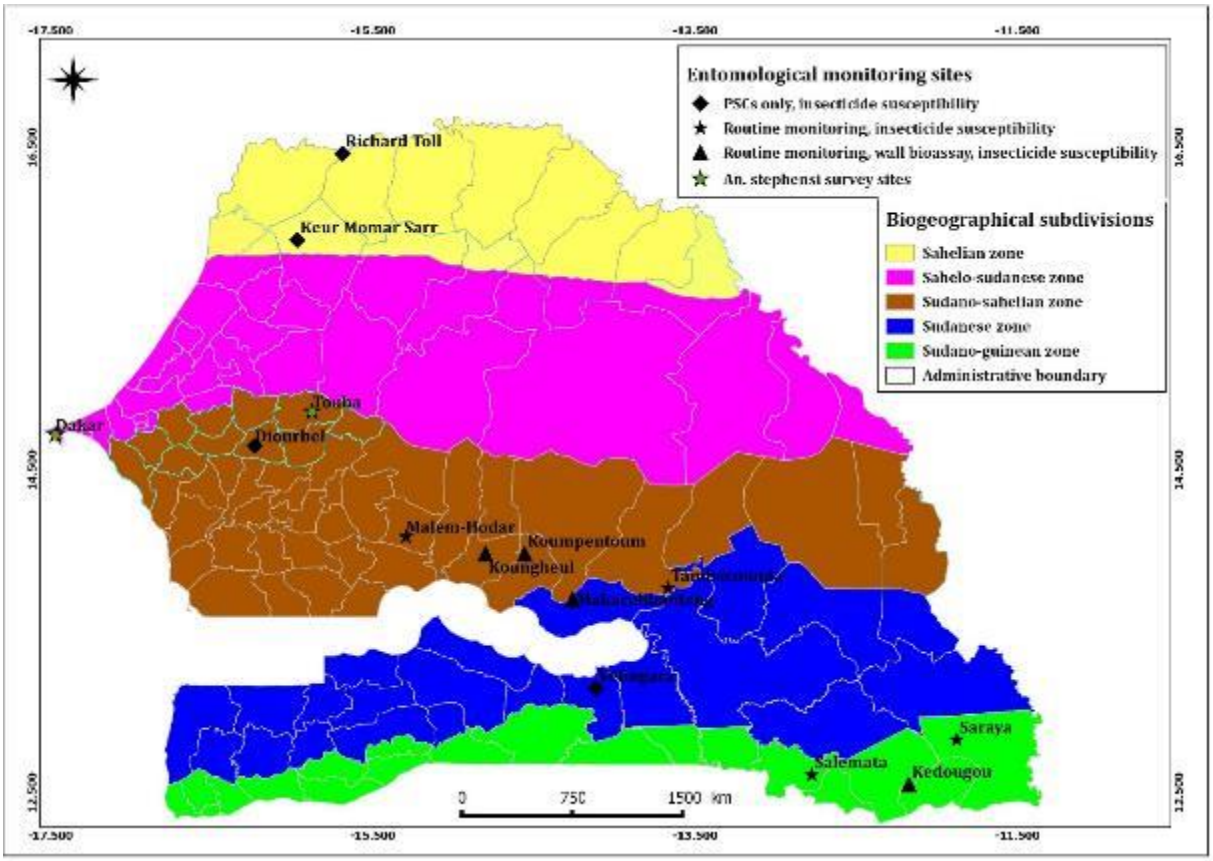


Table 1: Districts, Sites, Collection Methods and Frequency of Monitoring Activities

Geographical zone	Region	District*	# Site	HLC	PSC	ORC	CDC-LT (CBS Sites)	Wall Bioassay	Insecticide Susceptibility ****	*An. stephensi* survey
Sahelian	Saint Louis	Richard Toll	1		X		X			
	Louga	Keur Momar Sarr	1		X		X			
Sudano-Sahelian	Diourbel	Diourbel	1		X		X			
		Touba	1		X		X		X	X
	Kaffrine	Koungheul (IRS)	2	X	X			X	X	
		Malem Hodar (IRS Control)	2	X	X				X	
Sudanese	Tambacounda	Koumpentoum (IRS)	2	X	X			X	X	
		Makacolibantang (IRS)	2	X	X			X	X	
		Tambacounda **(PBO and Standard ITNs, IRS Control)	6	X	X	X***			X	
	Kolda	Velingara	2		X		X		X	
Sudano-Guinean	Kédougou	Kédougou (IRS)	2	X	X	X		X	X	
		Saraya (IRS Control)	1	X	X	X				
		Salemata (IRS Control)	1	X	X	X				
Sahelo-Sudanese	Dakar	District Sud	1							X
		District Centre	1							X
		Dakar Ouest	1						X	

*The vector control strategy in each district is standard ITNs unless indicated otherwise.
**Of the six sites in Tambacounda, two were selected as IRS controls and four for PBO ITN monitoring.
***ORC was conducted in four of the six Tambacounda sites.
****Insecticide susceptibility testing was done in one site per district, for a total of 10 sites. It was originally planned to be done in all districts but was not possible due to insufficient larvae found in some sites.
Key: HLCs=human landing catches, PSCs=pyrethroid spray catches, CBS=community-based surveillance, ORC=outdoor resting collection, CDC-LT=Centers for Disease Control and Prevention light trap.

2.2 Longitudinal Monitoring of Malaria Vectors

Adult mosquitoes were collected every month from April to December 2022, using pyrethrum spray catches (PSCs), human landing catches (HLCs), Centers for Disease Control and Prevention light traps (CDC-LTs), and outdoor resting collection (ORC) using Prokopack aspirators. All entomological data collections were conducted following PMI standard operating procedures.

Noted that rainy season duration varies in each geographical zone. It is from July to October in Sahelian, and Sudano-Sahelian and from June to November in Sudanese and Sudano-Guinean.

2.2.1 Pyrethrum Spray Catch (PSC)

PSCs were conducted in 10 selected houses from 7 a.m. to 9 a.m. in one day and once per month from April through December 2022. 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 the aerosol, 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), preserved in 1.5 ml Eppendorf tubes on silica gel, and kept in boxes at the laboratory for further species identification using the polymerase chain reaction (PCR) technique.

2.2.2 HUMAN LANDING CATCH (HLC)

HLCs were conducted indoors and outdoors in three houses per selected village during two consecutive nights. In all districts, two human volunteers (trained adult mosquito collectors) were positioned, one inside the house and the other outside at least five meters from the house, to collect mosquitoes. Collections were done 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 appropriate 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 on silica gel for subsequent molecular analysis.

2.2.3 OUTDOOR RESTING COLLECTION (ORC)

ORCs were performed using the Prokopack aspirators 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

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 a.m. to 9:00 a.m.	1 day per site per month	10 houses per site per month
HLC (Indoor and Outdoor)	8:00 p.m. to 6:00 a.m.	2 successive nights per site per month	3 houses per site
CDC LT (Indoor)	8:00 p.m. to 6:00 a.m.	2 successive nights per site per month	3 houses per site
ORC	7:00 a.m. to 9:00 a.m.	1 day per site per month	10 artificial shelters

Table 3: Entomological Indicators per Collection Method

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

2.2.4 COMMUNITY-BASED SURVEILLANCE (CBS)

The large number of sites to be surveyed is still a big challenge with a limited number of field technicians. To help better use and save resources as well as to decentralize surveillance efforts with more implication of the communities, VectorLink conducted a CBS pilot in six sentinel sites in 2022, which will be scaled up to seven additional sentinel sites in 2023.

The CBS approach was implemented in six sites (Keur Momar Sarr (1), Richard Toll (1), Diourbel (1), Touba (1) and Velingara (2)) located across three zones. Collections were carried out from August to December 2022. The collections were done in three houses per night for two nights per month using indoor baited CDC-LT, and 10 different houses per day per month using PSC.

2.2.5 *ANOPHELES STEPHENSI* SURVEY IN DAKAR AND IN TOUBA

Larval collections were conducted in *An. stephensi* potential habitats in Dakar and Touba to assess its presence. The larval habitats were selected following described habitats by previous reports (Sinka et al. 2020; Balkew et al. 2021). These sites are urban areas and have a dynamic and international maritime and air transport network. In Dakar, surveys were carried out around Leopold Sedar Senghor Airport (western district) and the autonomous port (southern district). In Touba, surveys were carried out in the heliport district and the large garage.

Two surveys were conducted in Dakar (in September and November 2022) and one in Touba **(**September 2022). In each site, all the water bodies encountered were geo-referenced and each water body visited. During each visit, parameters for characterization of the larval habitats were recorded (type of water body, area, depth, turbidity, exposure to the sun, etc.). The "dipping" method for larvae collection was used as described by Service (1993). The larvae were counted to estimate the larval density and then packaged in cups containing water for their breeding in the insectarium. The adults from these larvae were morphologically identified (Coetzee 2020) and then stored in individual tubes for molecular characterization of the species (Scott et al., 1993).

2.3 WHO Susceptibility Test

Susceptibility of adult *An. gambiae* s.l. was assessed against different insecticides using the standard WHO susceptibility test kits and WHO 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%, 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 WHO bottle assays (VectorLink Standard Operating Procedure 04/01). The susceptibility testing was conducted as described above and the mortality was recorded up to three days post exposure for chlorfenapyr and 24 hours for clothianidin. When insecticide resistance to pyrethroids was confirmed, resistance intensity (high, moderate, low) was also tested at 5× and 10× the diagnostic concentration of permethrin, deltamethrin, and alpha-cypermethrin, using the above WHO method.

Synergist assays with PBO (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).

2.4 Laboratory Analysis

Morphologically identified *An. gambiae* s.l. and *An. funestus* s.l. (Coetzee 2020) mosquitoes collected during HLCs, CDC-LTs**,** Prokopack aspirator, and PSCs longitudinal monitoring were preserved on silica gel prior to subsequent laboratory analyses. Both carcasses of the dissected vectors and non-dissected were sent to the laboratory for processing.

2.4.1 Molecular identification of *An. gambiae* s.l. / *An. funestus* group sub-species and characterization of target site resistance genes

Sibling species of a subsample of *An. gambiae* complex and *An. funestus* group collected both by HLCs and PSCs were identified using the PCR technique as described by Wilkins et al. (2006) and Koekemoer et al. (2002). Additionally, the mutations L1014S (*Kdr*-east) and L1014F (*Kdr*-west), responsible for resistance to pyrethroids and organochlorines, were investigated in the different *An. gambiae* s.l. populations that had been exposed to insecticides. The presence of the knock-down resistant west and east allele mutations and the acetylcholinesterase mutation (*Ace-1* [G119S]) were screened among dead and alive specimens exposed to insecticides as described by Huynh et al. (2007) and CDC-adapted Weill et al. (2004), respectively.

2.4.2 Origin of blood meals

The origin of blood meals was identified using the direct enzyme-linked immunosorbent assay (ELISA) method described by Beier et al. (1988) on blood-fed females collected by PSCs. The anthropophilic rate (AR) was calculated as the proportion of human blood among the total blood meals determined. The same formula was applied to estimate the host preference for the alternative animal hosts. Each host identified in the mixed meals was counted and included in the calculation of anthropophilic and zoophilic rates.

2.4.3 *PLASMODIUM FALCIPARUM* INFECTION RATE

The presence of *Plasmodium falciparum* circumsporozoite protein was characterized using the ELISA method (Burkot et al. 1984; Wirtz et al. 1989) to determine the infection rates among host-seeking females. Positives were not boiled but retested for confirmation following standard procedures. The sporozoite rate was calculated as the proportion of females found with the protein out of the total analyzed. The entomological inoculation rate (EIR) was calculated by multiplying the human biting rate by the sporozoite rate.

2.5 WALL BIOASSAYS

Four districts were sprayed during the 2022 Senegal IRS campaign conducted from May 30 to June 24, 2022. Kédougou was sprayed with SumiShield 50WG containing 50% w/w Water Dispersible Granule (300 mg ai/m^2); Koungheul and Koumpentoum with Fludora Fusion Wettable Powder containing 500 g/kg clothianidin+62.5 g/kg deltamethrin; and Makacolibantang with Actellic 300 SC containing micro-encapsulated formulation of pirimiphos-methyl 30.

The residual efficacy of insecticide-treated walls was assessed monthly using cone bioassays following the PMI VectorLink Standard Operating Procedure. Six houses in each of the two sprayed villages were randomly selected in each IRS district. Five of them were sprayed. 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, 1 m, and 1.5 m above the floor, and three cones at the control house. About 10 females, two to five days old, from the laboratory susceptible strain of *An. coluzzii* maintained in the insectary of the Laboratory of Vector and Parasite Ecology 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.

2.6 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 the Cheikh Anta Diop University (Université Cheikh Anta Diop [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 2022 was analyzed using VectorLink Collect. The platform includes comprehensive dashboards to synthesize vector bionomics and insecticide resistance summary results.

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 .05. The 95% confidence intervals were calculated for each *P. falciparum* infection rate.

VectorLink attempted to scale up mobile data collection in all sites in 2022. However, due to resource limitations (specifically availability of the UCAD database manager) and gaps in capacity, it was not fully scaled as planned. Scale-up is a priority under the new PMI Evolve project, with resources better allocated and a focus on capacity-building among UCAD staff.

3. RESULTS

3.1 VECTOR POPULATION DYNAMICS

3.1.1 SPECIES COMPOSITION PER GEOGRAPHICAL ZONE

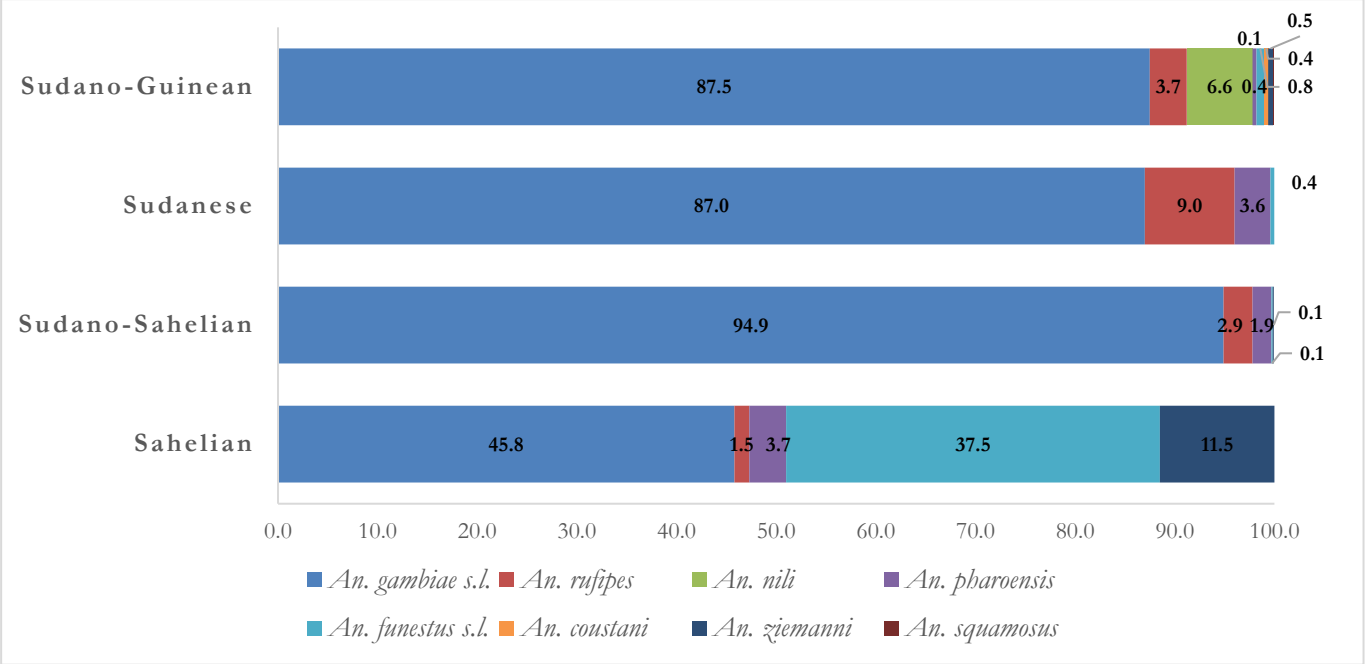
A total of 5,675 *Anopheles* mosquitoes, including eight different species (*An. gambiae* s.l., *An. funestus* s.l., *An. pharoensis*, *An. rufipes*, *An. nili*, *An. coustani, An. ziemanni and An. squamosus*), were collected from all sentinel districts of all geographical zones. *Anopheles gambiae* s.l. represented the main *Anopheles* and vector species collected (86.1%; n= 4,888), within the country, followed by *An. rufipes* (4.7%, n=266) and *An. nili* (3.7% n= 211). All eight *Anopheles* species were recorded in the Sudano-Guinean zone, including *An. squamosus* and *An. nili* that were found only in this zone. *Anopheles gambiae* s.l., *An. funestus* s.l., *An. pharoensis*, and *An. rufipes* were present in all the surveyed geographical zones. The Sudano-Guinean zone contributed to 56.0% of all *Anopheles* mosquitoes collected followed by the Sudanese zone with 24.4% (Figure 3, Table 4).

The site of Keur Momar Sarr in the Sahelian zone is close to the river and vegetation, which contributed particularly to the high density of *An. funestus* s.l. collected compared to all other zones (Annex A).

Table 4: *Anopheles* species composition collected across the country using HLC, CDC LT and PSC From April through December 2022

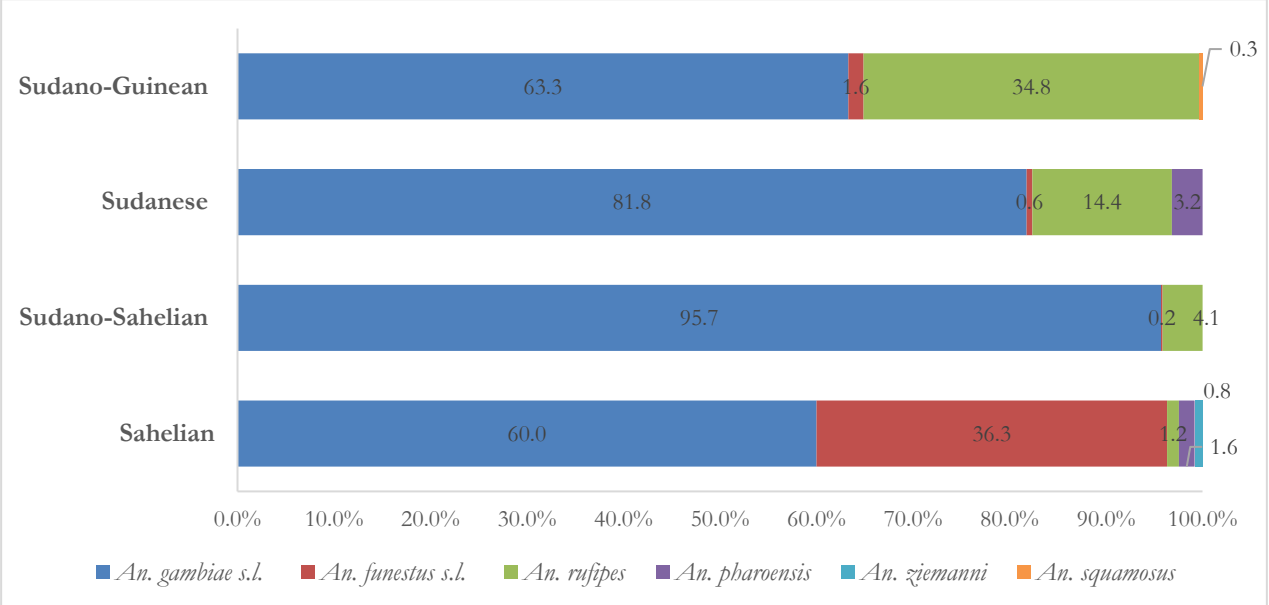
	Sahelian	Sudano-Sahelian	Sudanese	Sudano-Guinean	Total species	Percentage (%)
An. gambiae s.l.	148	805	1155	2,780	4,888	**86.1**
An. funestus s.l.	121	1	5	24	151	**2.7**
An. rufipes	5	25	120	116	266	**4.7**
An. pharoensis	12	16	48	14	90	**1.6**
An. nili	0	0	0	211	211	**3.7**
An. coustani	0	0	0	13	13	**0.2**
An. ziemanni	37	1	0	16	54	**1.0**
An. squamosus	0	0	0	2	2	**0.0**
Total species	**323**	**848**	**1328**	**3,176**	**5,675**	
Percentage (%)	5.7	14.9	23.4	56.0		

Figure 3: *Anopheles* Species Composition by Geographical Zone Collected Using HLC, PSC, and CDC-LT from April through December 2022



Using PSC, a total of 1,983 *Anopheles* mosquitoes were collected. *An. nili* and *An. coustani* were not collected using PSC. *Anopheles rufipes* (12.9; n=256) was the second species collected after *An. gambiae* s.l. (80.4%; n=1,594) (Figure 4). The other species collected were *An. funestus* (5.0%), *An. pharoensis* (1.5%), *An. ziemanni* (0.3%) and *An. squamosus* (0.2%).

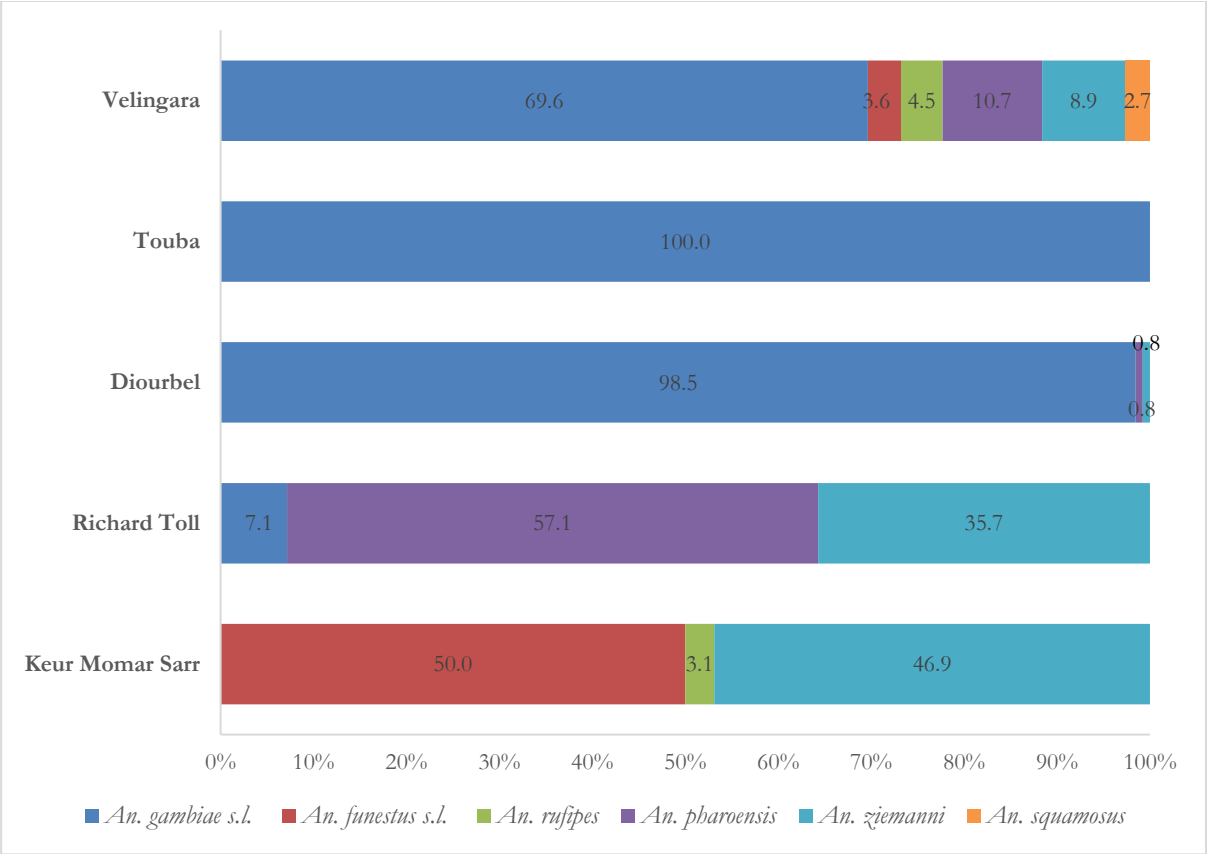
Figure 4: *Anopheles* Species Composition by Geographical Zone Collected Using PSC from April through December 2022



343 *Anopheles* mosquitoes were collected by CDC-LT in the six sentinel sites selected for CBS from September to December 2023. In Sahelian (Keur Momar Sarr and Richard Toll), *An. ziemanni* (44.9%, n= 35) and *An.*

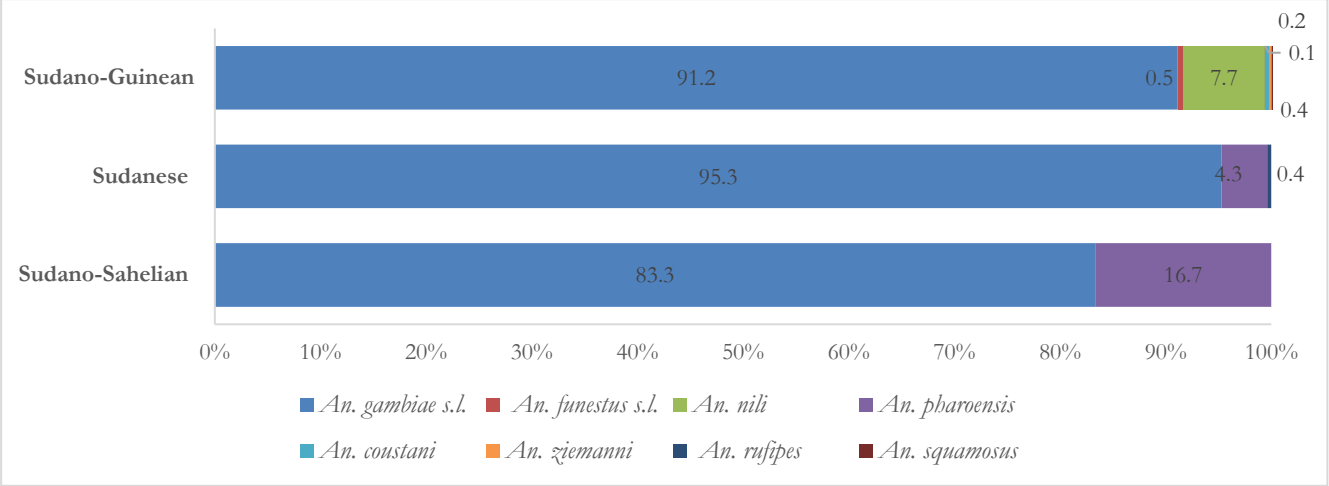
funestus (41.0%, n= 32) were predominant and few *An. gambiae* s.l. (1.3%, n= 4) were collected (Annex A3). In Sudano-Sahelian zone (Touba and Diourbel), *An. gambiae* represented 98.7% followed by *An. pharoensis* (0.7%) and *An. ziemanni* (0.7%). In Sudano Guinean zone (Velingara), six of the anopheles collected by CDC-LT were present with *An. gambiae* s.l. being predominant. (69.6%) (Figure 5 and Annex Table A3).

Figure 5: *Anopheles* Species Composition Collected Using CDC-LT, in CBS Sites, by District, from September through December 2022



A total of 3,349 *Anopheles* were collected using HLC from April to December 2023 across 18 sites. *An. gambiae* s.l. was predominant in all zones (83.3% in Sudano-Sahelian, 95.3% in Sudanese and 91.1% in Sudano-Guinean). The second species collected after *An. gambiae* s.l. was *An. pharoensis* in Sudano- Sahelian (16.7%) and Sudanese zone (4.3%) and *An. nili* (7.7%) in Sudano-Guinean zone. *An. rufipes* and *An. squamosus* were collected but less represented. (Figure 4). For mosquitoes collected through HLC, the Sudano-Guinean part of the country yielded the highest *Anopheles* species diversity. *Anopheles nili* and *An. squamosus* were only found in this part of the country (Figure 6).

Figure 6: *Anopheles* Species Composition by Geographical Zone Collected Using HLC from April through December 2022

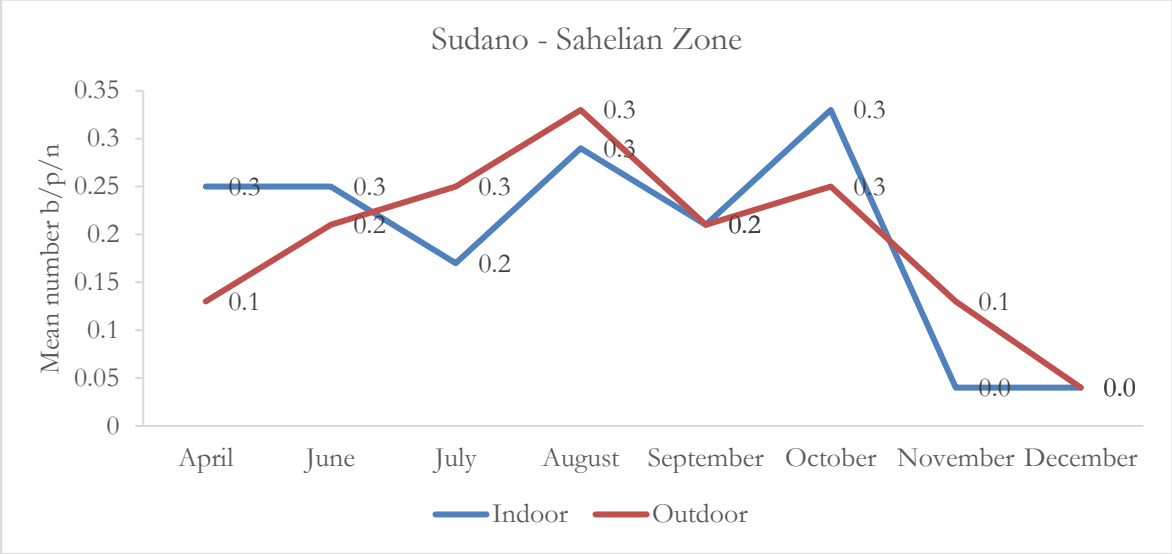


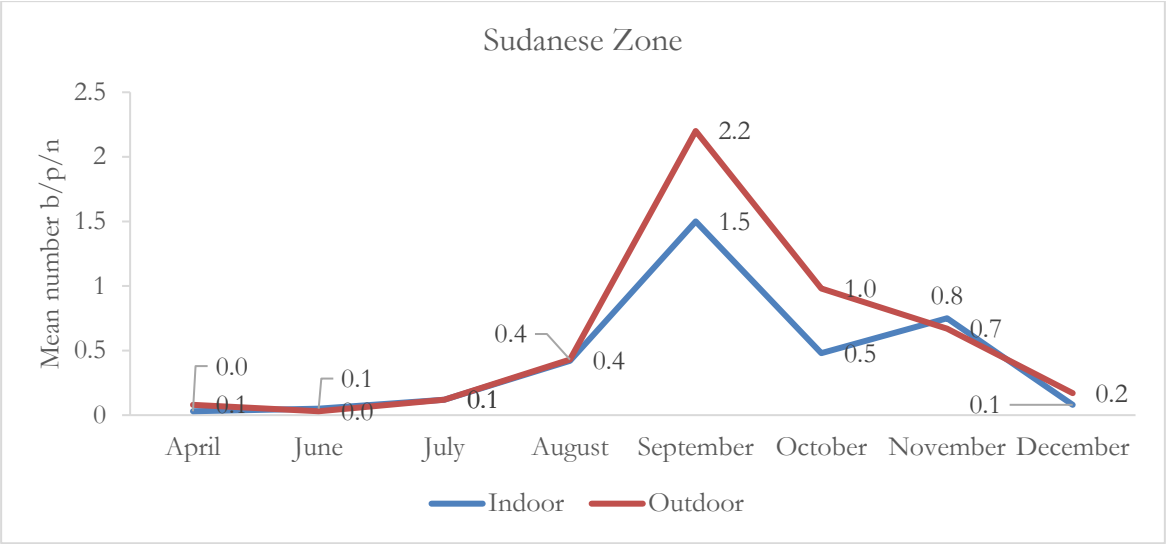
3.1.2 HUMAN BITING RATE AND VECTOR BITING BEHAVIOR BY GEOGRAPHICAL ZONE

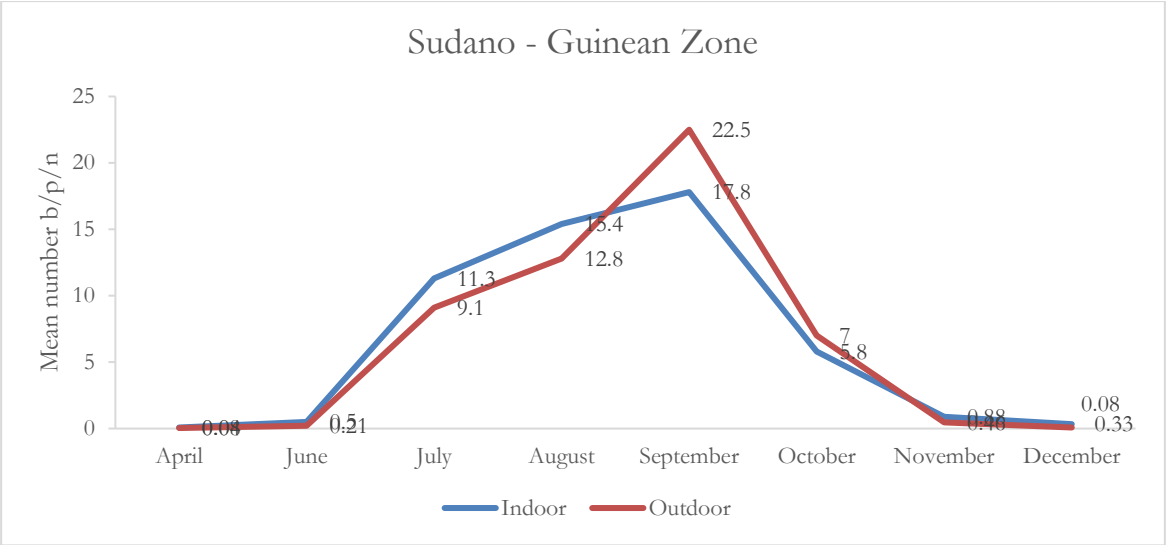
3.1.2.1 HUMAN BITING RATE OF *AN. GAMBIAE* S.L.

A total of 3,064 *An. gambiae* s.l. were collected using HLC at 18 sites. The average HBR at country level was 3.2 b/p/n. HBRs were highest in the Sudano-Guinean (10.7 b/p/n) and less in Sudano-Sahelian (1.0 b/p/n) zones. (Annex 1, Table A.2). The biting cycle was recorded in three of the zones surveyed. The indoor biting rates seemed to be similar to the outdoor biting rates in Sudano-Guinean Zone but less in Sudanese and Sudano-Sahelian zones (Annex A). Overall, the highest HBRs were recorded between August and September 2022 within all geographical areas except in Sudano-Sahelian zone where the highest HBRs were recorded in October (Figure 7), coinciding with the rainy season within the country.

Figure 7: Monthly Variation of *An. gambiae* s.l. Biting Rate by Geographical Zone



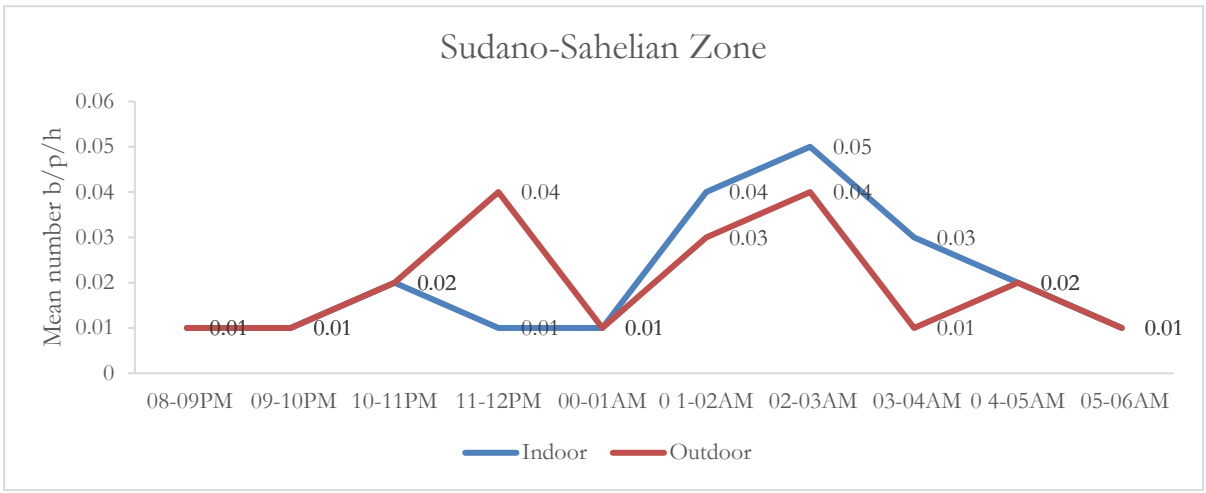


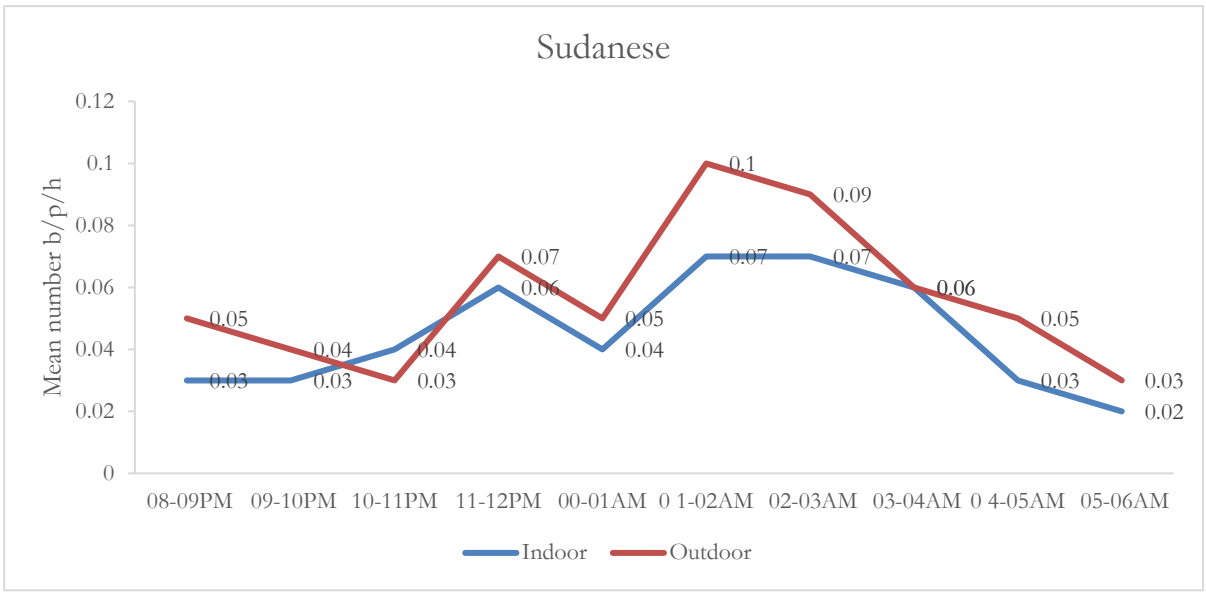


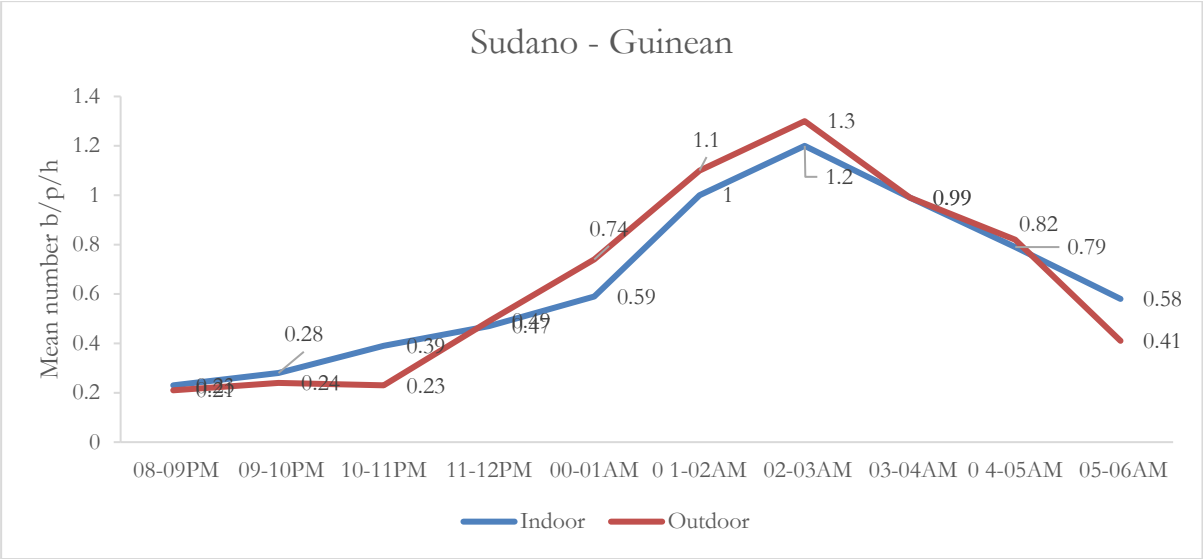
3.1.2.2 BITING TIME OF *AN. GAMBIAE* S.L.

In the Sudano-Sahelian zone, the mean hourly biting started rising at 11 PM in outdoor collection and from 1 AM to 2 AM in both indoor and outdoor collections. In Sudanese zone, HBR started increasing during the second half of the night with a peak at 1 AM, both indoors and outdoors. The highest peak hourly biting was recorded between 1 and 3 AM in Sudano-Guinean zone. Furthermore, the mean hourly biting rates were higher both indoors and outdoors in the Sudano-Guinean zone, with 1.3 indoor and 1.1 outdoor bites/person/hour occurring between 2 and 3 AM. In contrast, the Sudano-Sahelian zone recorded less than 1 bite/person/hour (0.54) and less than 1.5 bite/person/hour in the Sudanese zones, throughout the night, both indoors and outdoors (Figure 8).

Figure 8: Mean Hourly Biting Rate of *An. gambiae* s.l. by Geographical Zone



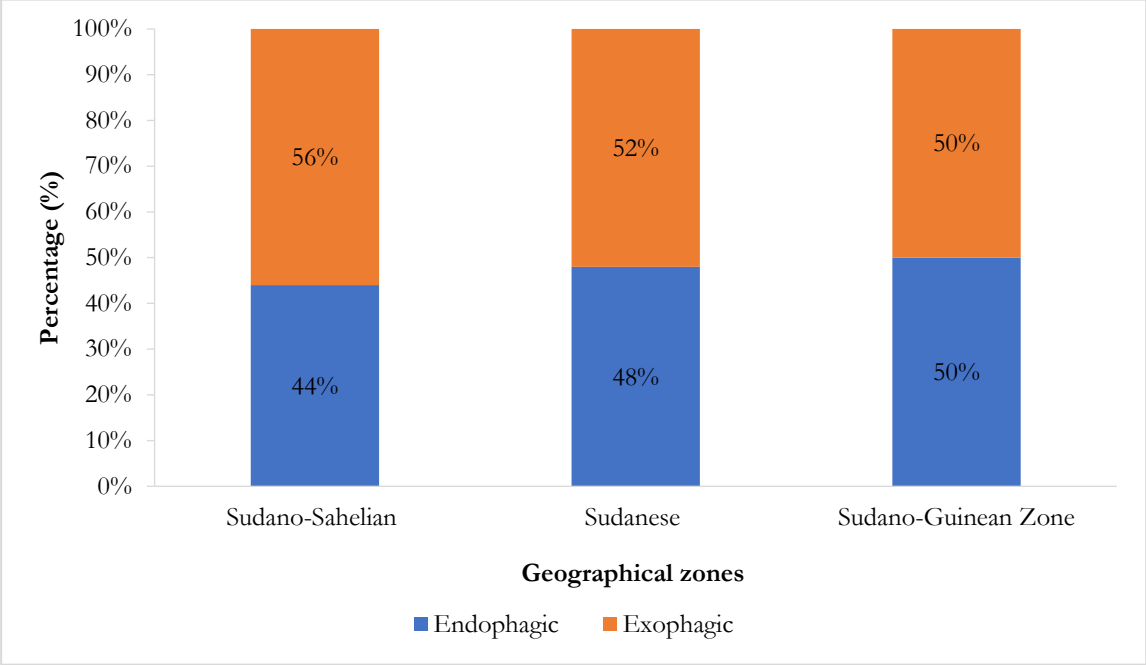




3.1.2.3 ENDOPHAGIC RATE OF *AN. GAMBIAE* S.L.

The mean endophagic rate of all surveyed sites within all geographical zones was estimated to 0.49 (Annex A), 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 zone, slightly higher in outdoor in the Sudanese zone (52% outdoor) and higher in Sudano-Sahelian zone (57% outdoor) (Figure 9).

Figure 9: Endophagic Rate of *An. gambiae* s.l. by Geographical Zone



3.1.3 INDOOR RESTING DENSITY AND ABDOMINAL STATUS OF FEMALE VECTORS BY GEOGRAPHICAL ZONE COLLECTED USING PSCS

3.1.3.1 *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 0.84 f/r for the whole monitoring period. The highest IRDs were recorded in Sudano-Sahelian (1.23 f/r) zone, while the lowest IRDs were recorded in the Sudano-Guinean (0.42 f/r) zone (Figure 10; Annex Table A3).

The proportion of blood-fed females found inside houses was significantly lower in the Sudanese zone (36.3% of the total collected in the Sudanese zone) ($p=0.001$) when compared with in other zones (Figure 9). The highest proportion of blood-fed females was recorded in the Sudano-Guinean zone at 60% of the total collected, followed by the Sahelian (56.5%) and Sudano-Sahelian (54.6%) zones (Figure 11).

Figure 10: Overall Monthly Indoor Resting Density of *An. gambiae* s.l. by Geographical Zone

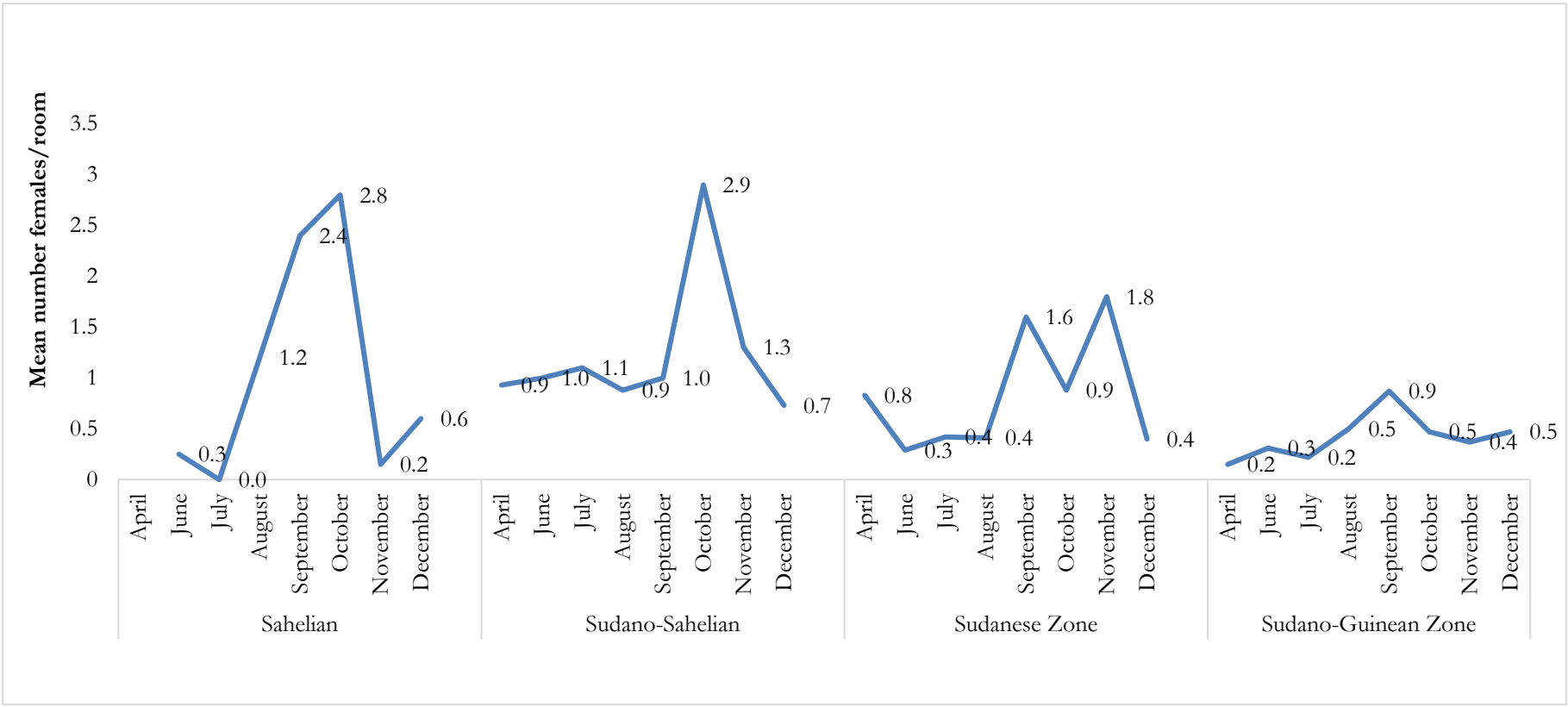
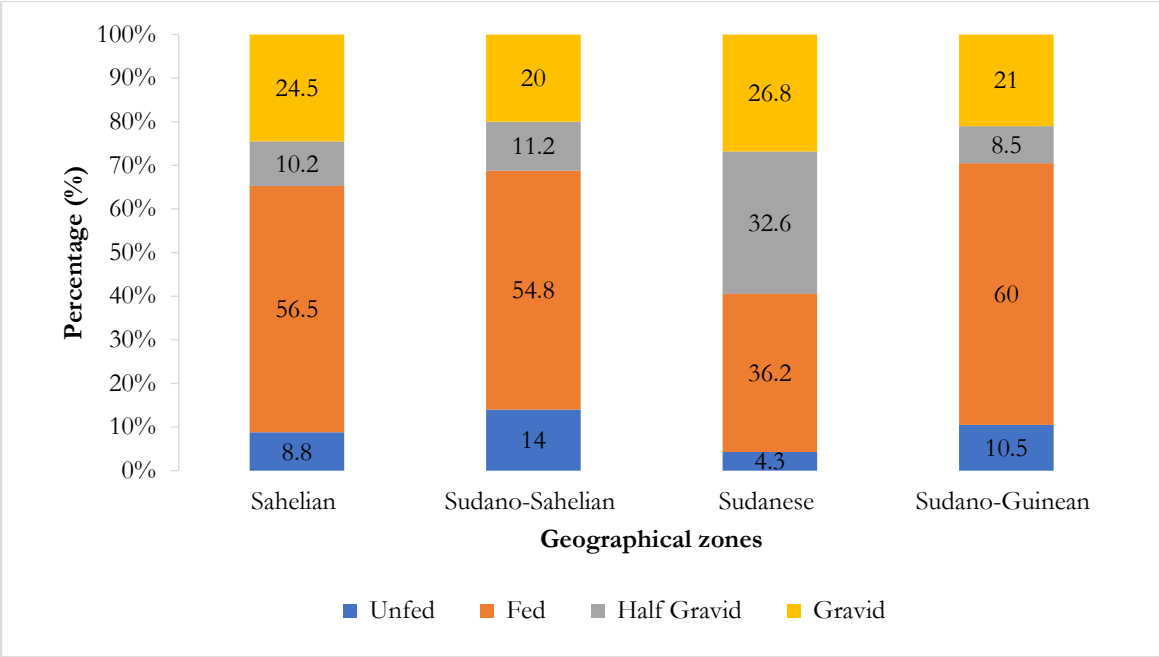


Figure 11: Abdominal Status of Female *An. gambiae* s.l. by Geographical Zone



3.1.4 OUTDOOR RESTING DENSITY AND ABDOMINAL STATUS OF FEMALE VECTORS COLLECTED USING PROKOPACK

Few *Anopheles* were collected outdoors through Prokopack aspiration in all surveyed areas (Table 5). Those collected included three diverse species (*An. gambiae* s.l., *An. funestus* s.l. and *An. rufipes*). *An. gambiae* s.l. was mostly found in the Sudanese zone (Table 5). In the Sudano-Guinean zone, only five *Anopheles* were collected in one site. All other sites did not record any specimen (Table 5). Gravid and semi-gravid females represented more than 50% of collected *An. gambiae* s.l. in Sudanese zone (Table 6).

Table 5: *Anopheles* Species Composition Collected Outdoors with Prokopack (April to December 2022)

Geographic zone	District	Locality	*An. gambiae* s.l.	*An. funestus* s.l.	*An. rufipes*	TOTAL
Sudanese	Tambacounda	Kouthia Fari Ndella	12	0	1	13
		Velingara Sabaké	3	0	0	3
		Oundoundou	5	0	0	5
		Safalou 1	9	0	0	9
	Sub total		**29**	**0**	**1**	**30**
Sudano-Guinean	Saraya	Bembou	1	3	1	5
	Salemata	Diara Pont	0	0	0	0
	Kédougou	Tomboronkoto	0	0	0	0
		Bandafassi	0	0	0	0
	Sub total		**1**	**3**	**1**	**5**
TOTAL			**30 (85.7%)**	**3 (8.6%)**	**2 (5.7%)**	**35**

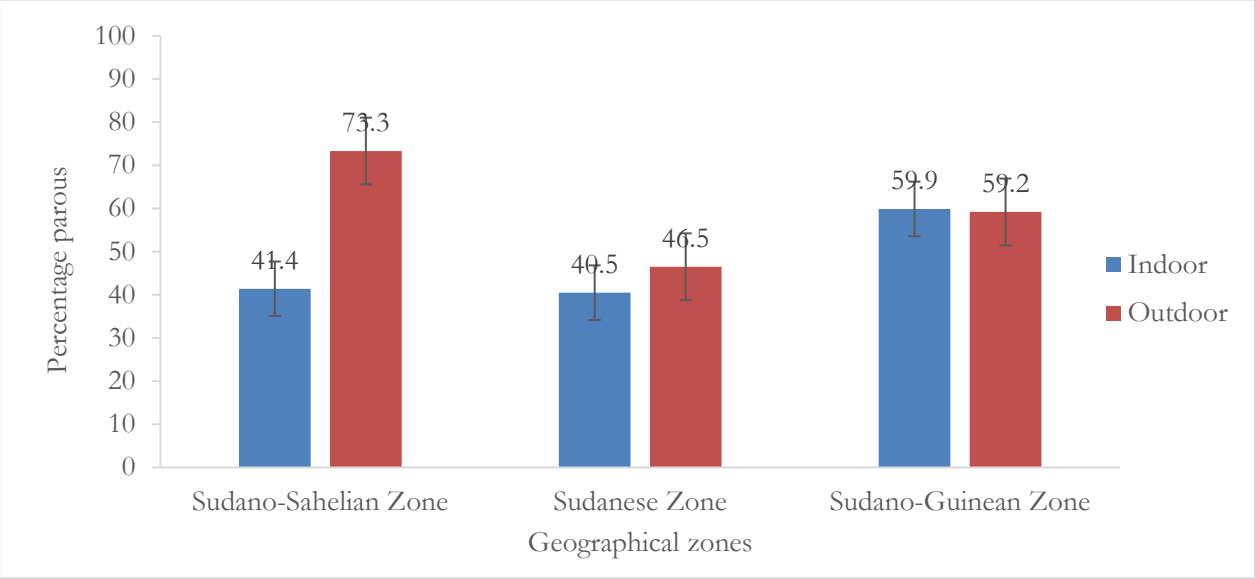
Table 6: Abdominal Status of *Anopheles* Collected Outdoors with Prokopack (April to December 2022)

Geographic zone	District	Locality	TC	Unfed	Fed	Gravid	Semi gravid
Sudanese	Tambacounda	Kouthia Fari Ndella	13	0	5	5	3
		Velingara Sabaké	3	0	1	1	1
		Oundoundou	5	0	2	2	1
		Safalou 1	9	1	3	3	2
	Sub total		**30**	**1 (3%)**	**11 (37%)**	**11 (37%)**	**7 (23%)**
Sudano-Guinean	Saraya	Bembou	5	2	3	0	0
	Salemata	Diara Pont	0	0	0	0	0
	Kédougou	Tomboronkoto	0	0	0	0	0
		Bandafassi	0	0	0	0	0
	Sub total		**5**	**2**	**3**	**0**	**0**
TOTAL			**35**	**3 (9%)**	**14 (40%)**	**11 (31%)**	**7 (20%)**

3.1.5 PARITY RATE

The highest parity rates were recorded from outdoor collections in the Sudanese-Sahelian zones (73.3%) and the lowest were recorded from indoor collections in the Sudanese zone at 40.5% (Figure 12).

Figure 12: *An. gambiae* s.l. Parity Rate by Geographical Zone



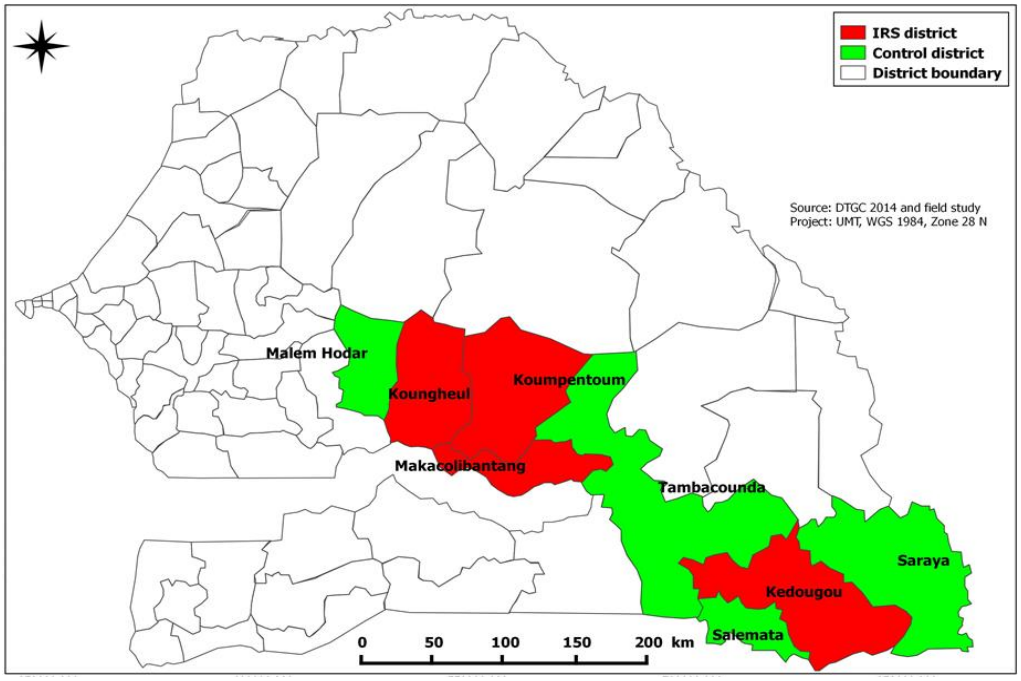
*Error bars represent the standard errors

3.2 ENTOMOLOGICAL INDICATORS OF IRS AND CONTROL SITES

3.2.1 IRS AND CONTROL SITE LOCATION

Longitudinal entomological monitoring using HLCs and PSCs was conducted in the four IRS sites (SumiShield in Kédougou, Actellic in Makacolibantang and Fludora Fusion in Koumpentoum and Koungheul) and control sites (Saraya, Salémata, Tambacounda, and Malam Hodar) (Figure 13).

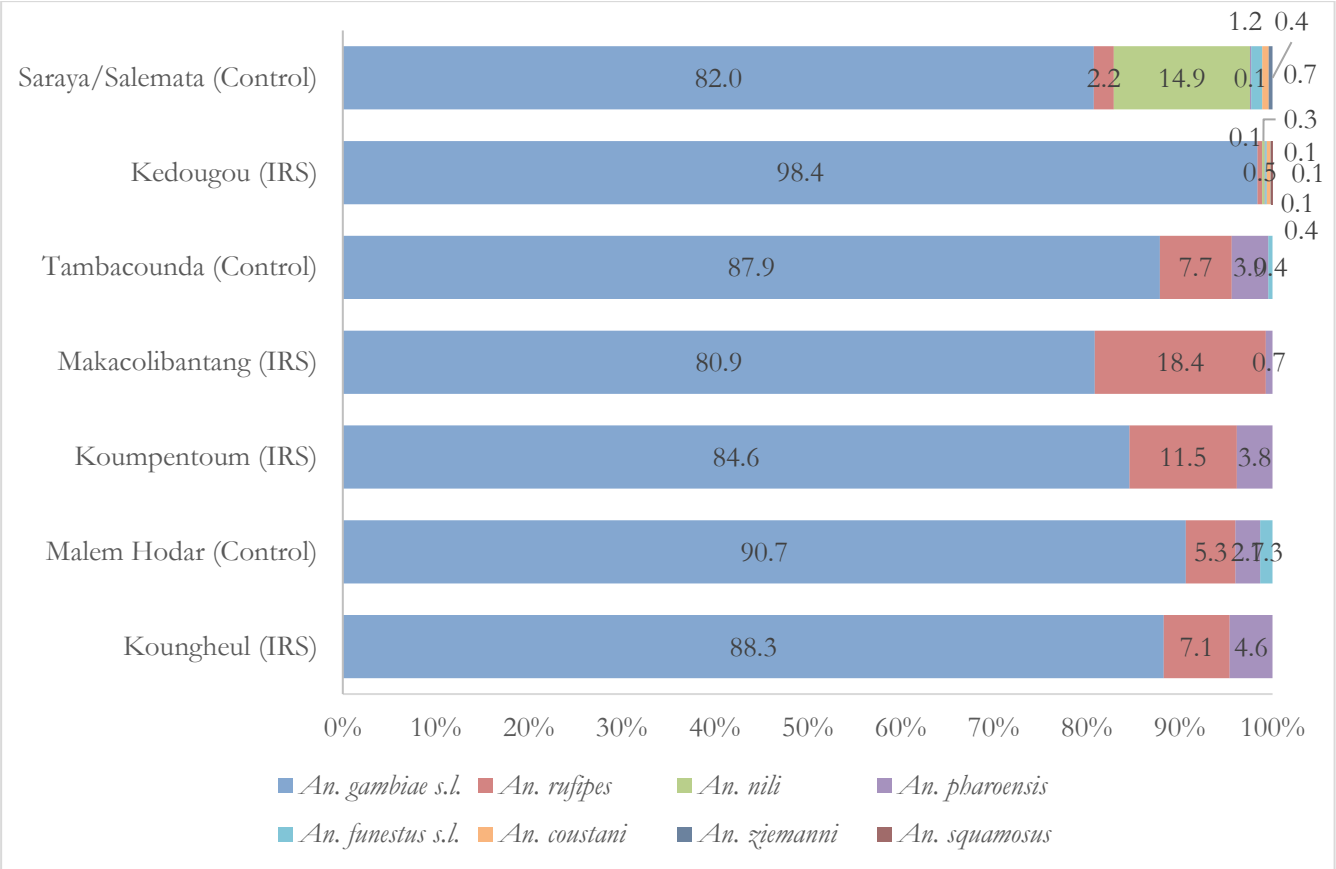
Figure 13: Map of the IRS and Control Sites



3.2.2 SPECIES COMPOSITION

In all IRS sites, *An. gambiae* s.l. was the main vector, representing 89.1% of the total *Anopheles* collected through HLCs and PSCs. *Anopheles rufipes* were collected in the four IRS districts and in their control sites where it represented the second most frequently collected *Anopheles* species (3.87%) except in the two control sites of Kedougou (Saraya and Salemata) where *An. nili* was the second *Anopheles* species collected with 14.9%. The highest diversity of *Anopheles* was recorded in Kédougou, with eight species collected (Figure 14; Annex Table A4).

Figure 14: *Anopheles* Species Composition in IRS Sites and Controls

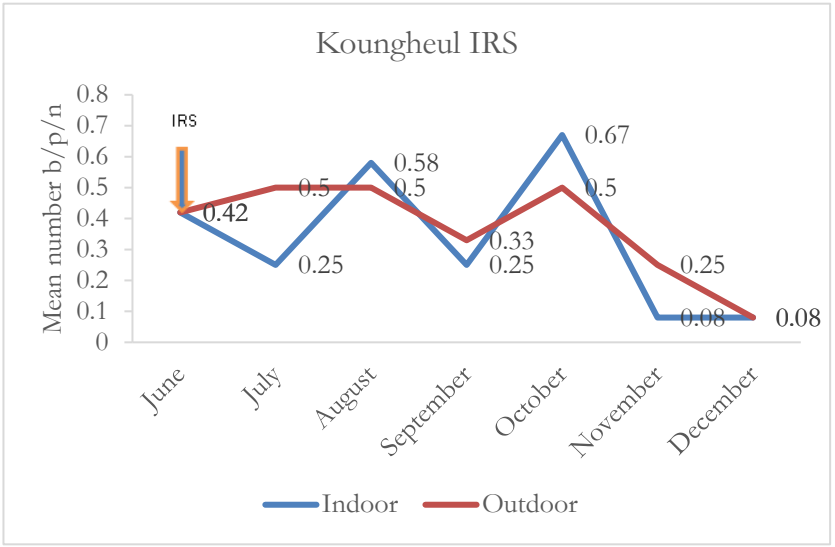


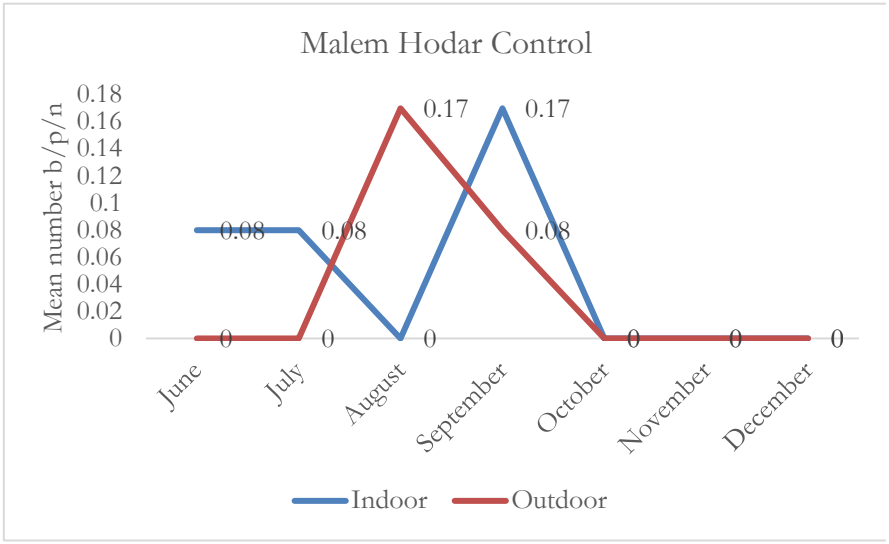
3.2.3 HUMAN BITING RATE AND VECTOR BEHAVIOR IN IRS AND CONTROL SITES

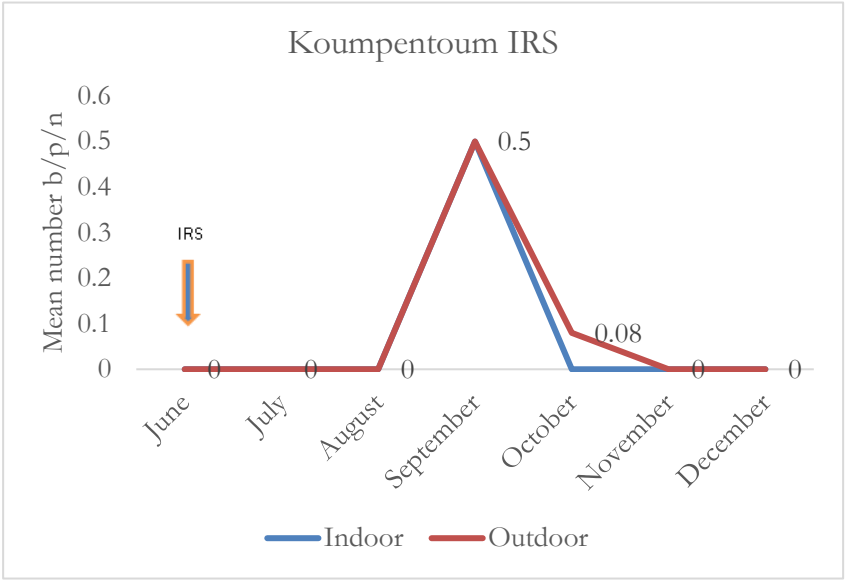
At all IRS sites, *An. gambiae* s.l. bit more indoors than outdoors except in Koumpentoum where the trends were similar in both outdoor and indoor settings. In control sites, outdoor biting rates were higher than indoor in Tambacounda and Salemata but similar in Malem Hodar (Figure 15). The overall mean indoor (10.8 b/p/n) and outdoor (6.5 b/p/n) HBR in the SumiShield sprayed sites of Kédougou was higher than that recorded in the Fludora Fusion sites of Koumpentoum and Koungheul and in the Actellic sites of Makacolibantang (≤ 0.7 b/p/n) (Annex Table A5). The peak indoor/outdoor biting was recorded in September in Koumpentoum and Kédougou, in October in Koungheul and in November in Makacolibantang. (Figure 15).

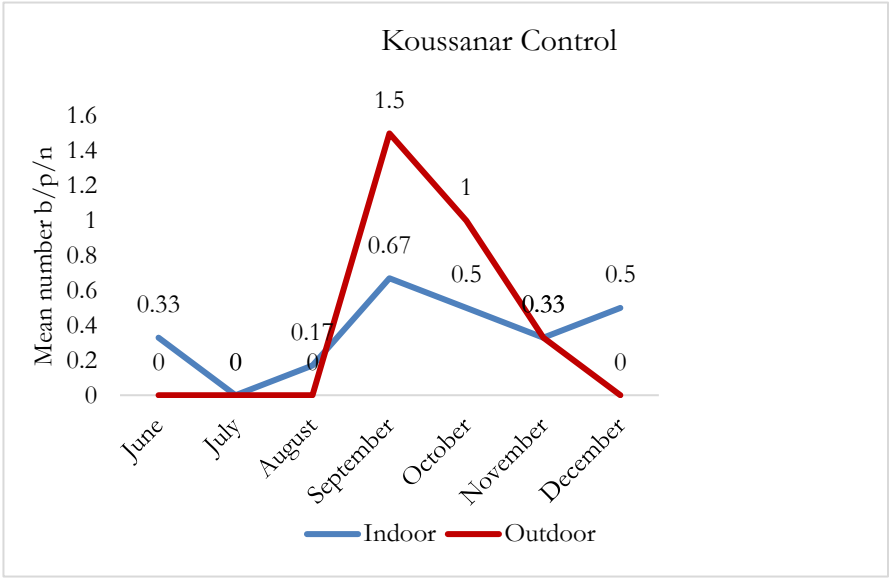
The HBR and vector behavior observed in the control districts varied with higher outdoor densities in Tambacounda and less outdoor densities in Salemata. The peak biting was observed between August and October 2022 in Malem Hodar and Tambacounda and between July to August in Salemata and Saraya (Figure 15).

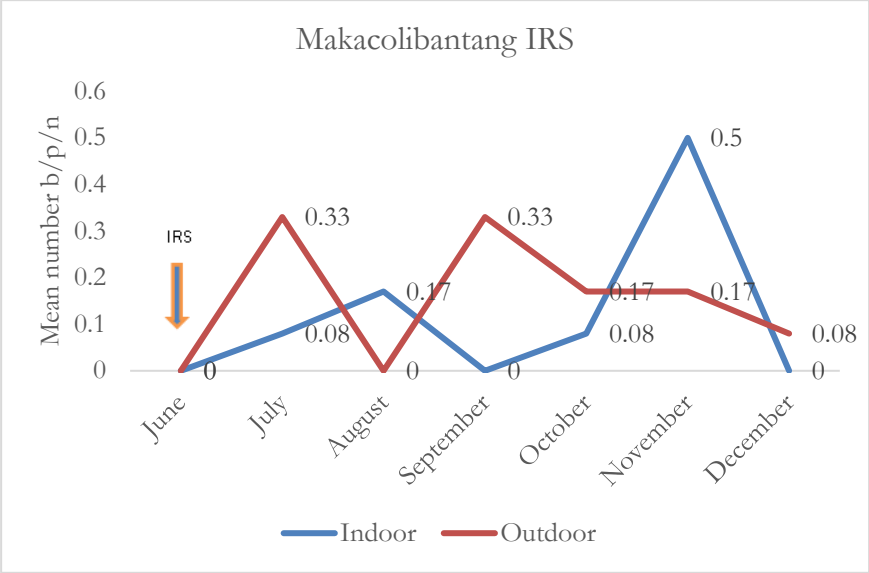
Figure 15: Indoor and Outdoor HBR of *An. gambiae* s.l. in IRS Districts and Their Controls

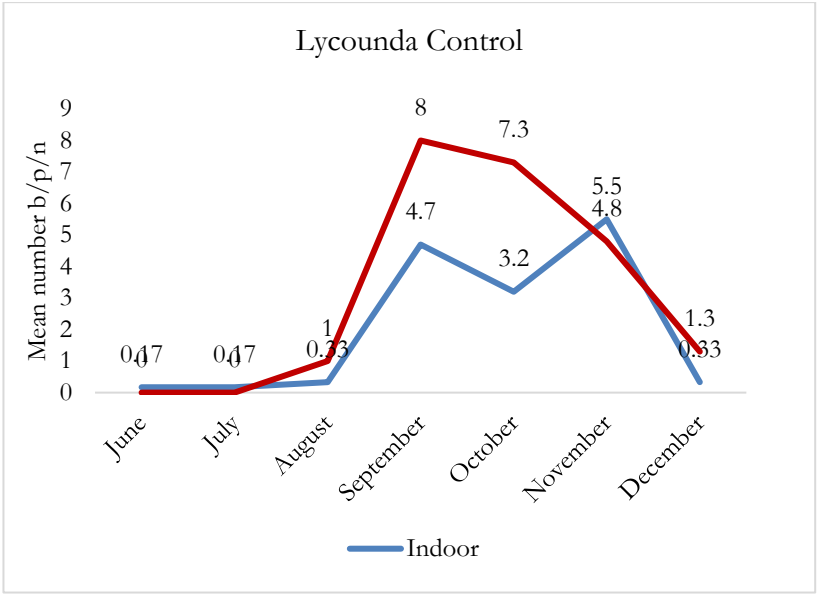


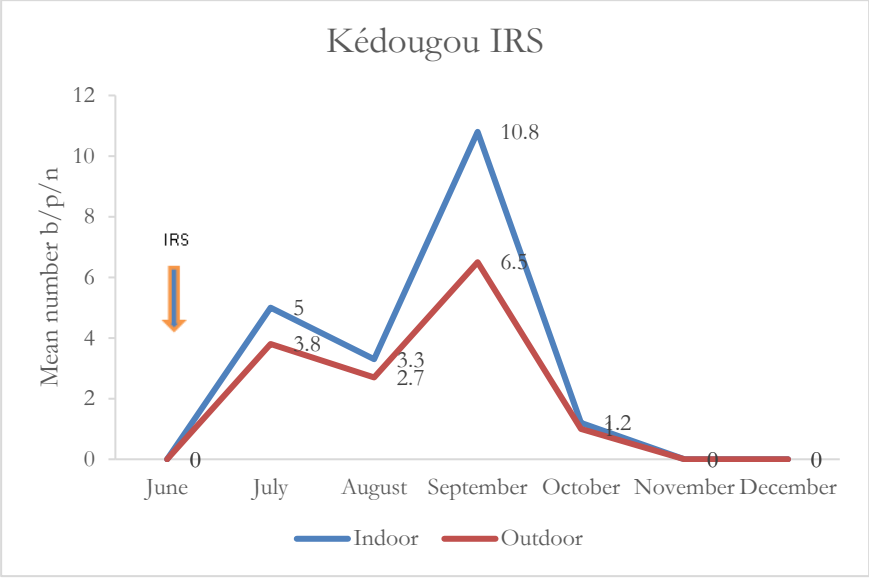


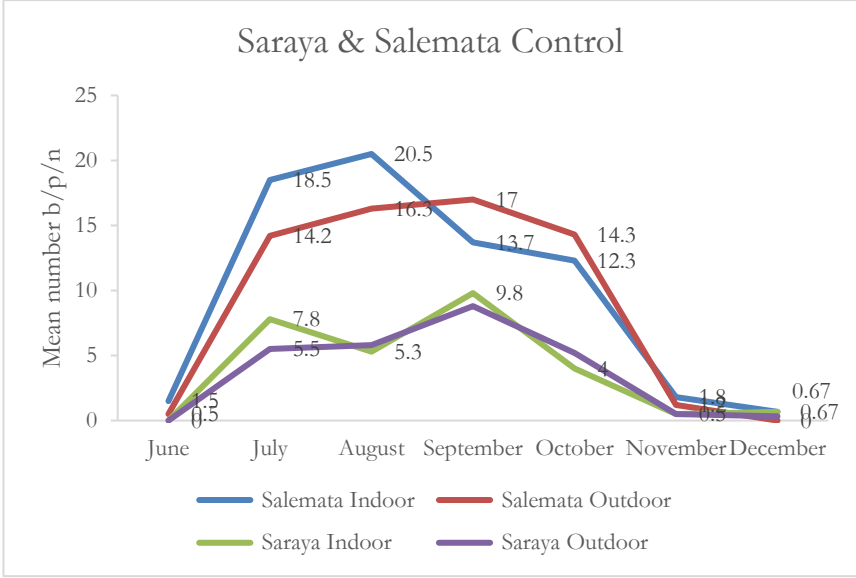





Makacolibantang IRS
Mean number b/p/n
0.6
0.5
0.4
0.3
0.2
0.1
0
IRS
0.33
0.33
0.5
0.17
0.17
0.17
0.08
0.08
0.08
0
0
0
0
0
June
July
August
September
October
November
December
Indoor
Outdoor


Lycounda Control
Mean number b/p/n
9
8
7
6
5
4
3
2
1
0
8
7.3
5.5
4.8
4.7
3.2
1
1.3
0.33
0.33
0.17
0.17
0
0
June
July
August
September
October
November
December
Indoor


Kédougou IRS
Mean number b/p/n
12
10
8
6
4
2
0
IRS
10.8
6.5
5
3.8
3.3
2.7
1.2
0
0
0
June
July
August
September
October
November
December
Indoor
Outdoor


Saraya & Salemata Control
Mean number b/p/n
25
20
15
10
5
0
20.5
18.5
17
16.5
14.2
14.3
13.7
12.3
9.8
7.8
5.5
5.3
4
1.8
0.67
0.5
0.5
0
0
June
July
August
September
October
November
December
Salemata Indoor
Salemata Outdoor
Saraya Indoor
Saraya Outdoor

3.2.4 *AN. GAMBIAE* S.L. PARITY RATE IN IRS AND CONTROL SITES

A total of 1,482 *An. gambiae* s.l. across IRS sites and 1,582 in controls sites were ovary-dissected for parity rates, of which 52% and 60% (respectively) were parous. The mean parity rates in both IRS sites and control sites were 57% (Table 7). Only HLC collected mosquitoes were dissected for parity, including both from indoors and outdoors, which had similar parity rates (Table 7).

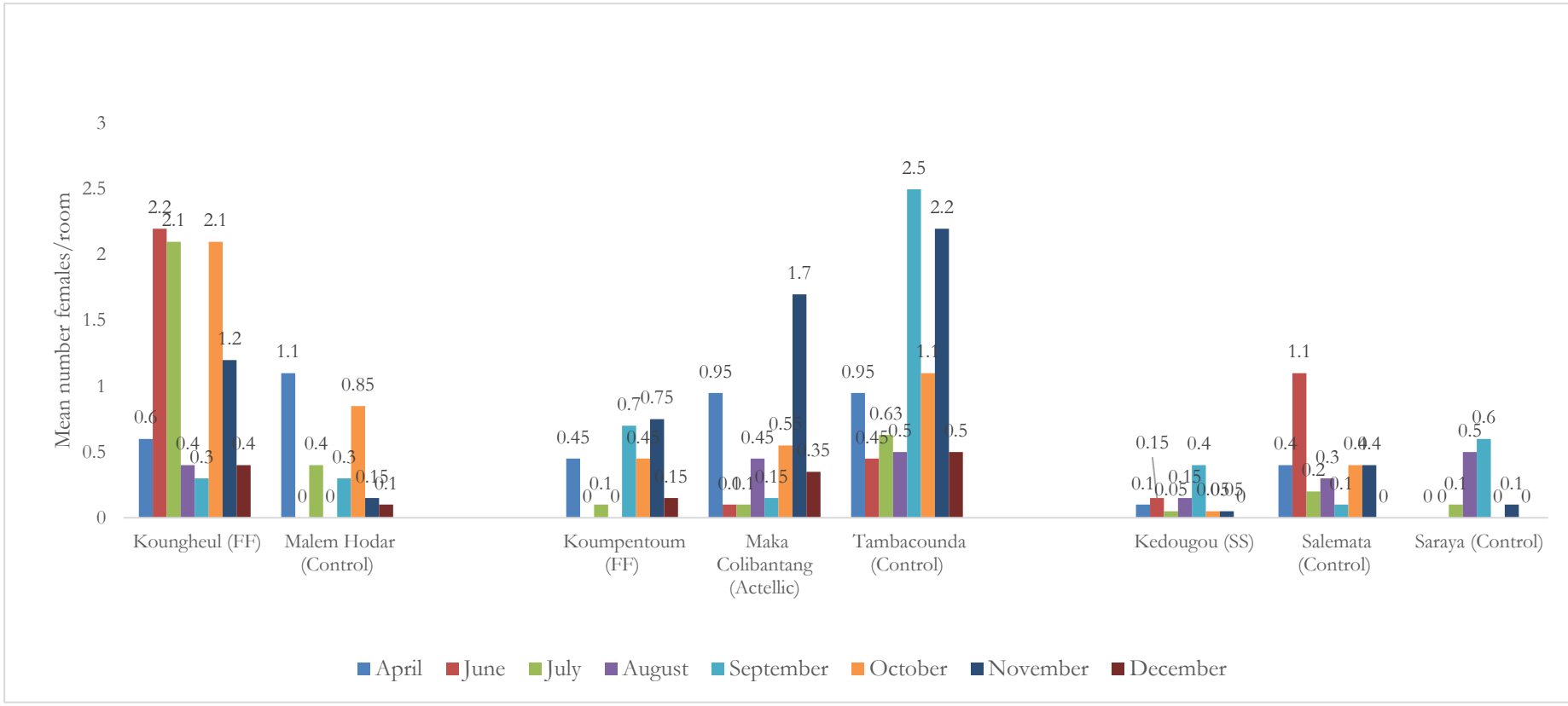
Table 7: Parity Rate of *An. gambiae* s.l. in IRS Districts and Controls

			Indoor				Outdoor				Total			
Insecticide	**Intervention**	**District**	**Collected**	**Dissected**	**Parous**	**% Parous**	**Collected**	**Dissected**	**Parous**	**% Parous**	**Collected**	**Dissected**	**Parous**	**% Parous**
Fludora Fusion	IRS	Koungheul	31	25	12	**48.0**	34	27	20	**74.1**	65	52	32	**61.5**
	Control	Malem Hodar	7	4	0	**0.0**	3	3	3	**100.0**	10	7	3	**42.9**
	IRS	Koumpentoum	7	6	0	**0.0**	7	7	0	**0.0**	14	13	0	**0.0**
Actellic	IRS	Makacolibantang	10	1	0	**0.0**	13	4	2	**50.0**	23	5	2	**40.0**
	Control	Tambacounda	190	156	66	**42.3**	260	202	97	**48.0**	450	358	163	**45.5**
SumiShield	IRS	Kédougou	666	453	231	**51.0**	714	411	220	**53.5**	1380	864	451	**52.2**
	Control	Salémata	415	379	265	**70.0**	381	321	208	**64.8**	796	700	473	**67.6**
	Control	Saraya	169	147	90	**61.2**	157	141	89	**63.1**	326	288	179	**62.1**
	Subtotal Salemata & Saraya Control		**584**	**526**	**355**	**67.5**	**538**	**462**	**297**	**64.3**	**1122**	**988**	**652**	**66.0**
Total IRS districts			714	485	243	**50.1**	768	449	242	**53.9**	1482	934	485	**51.9**
Total Control districts			781	686	421	**61.4**	801	667	397	**59.5**	1582	1353	818	**60.5**
Total IRS and Controls			**1495**	**1171**	**664**	**56.7**	**1569**	**1116**	**639**	**57.3**	**3064**	**2287**	**1303**	**57.0**

3.2.5 INDOOR RESTING DENSITY OF *AN. GAMBIAE* S.L. IN IRS DISTRICTS AND CONTROLS

A total of 1,360 rooms were sprayed across the eight IRS and six control sites. Koungheul and Koumpentoum recorded the highest IRD (1.18 f/r) among the IRS sites, while Tambacounda yielded the highest IRD (1.1 f/r) among the different control sites (Figure 16; Annex Table A6).

Figure 16: IRD *of An. gambiae* s.l. in IRS Districts and Controls



3.3 MALARIA VECTOR SUSCEPTIBILITY TO INSECTICIDES

WHO insecticide susceptibility tests were carried out only against *An. gambiae* s.l. in the surveyed sites that had sufficient larvae. WHO bottle assays were also conducted in selected sites, using chlorfenapyr and clothianidin insecticides. Of the 15 districts where insecticide susceptibility testing was planned, testing was completed in 10 districts. All the sites could not be surveyed, and all insecticides were not tested at all sites due to limited number of larvae. Annex C presents the sites where insecticide susceptibility testing was completed.

3.3.1 SUSCEPTIBILITY AND SYNERGIST ASSAYS

Pyrethroid resistance was observed in all sites and for all insecticides except deltamethrin in Koungheul where the resistance was suspected (91%). The synergist assay test was conducted in nine districts including IRS and PBO-ITN areas (Figure 17). With deltamethrin, pre-exposure to PBO reversed the resistance status of the *An. gambiae* s.l. population of Koungheul, Koumpentoum, Makacolibantang and Tambacounda to susceptibility, while a partial increment of mortality was recorded in Keur Massar, Touba, Velingara and Kédougou. PBO + permethrin tested in seven of the districts also reversed the resistance status of the *An. gambiae* s.l. population only in Koungheul, and Koumpentoum and yielded a partial mortality increment in Touba and Kédougou. With alpha-cypermethrin tested in nine districts, pre-exposure to PBO reversed the resistance status of the *An. gambiae* s.l. population of Koungheul, Koumpentoum and yielded a partial mortality increase in Keur Massar, Touba, Tambacounda, Makacolibantang and Kédougou (Figure 17).

3.3.2 INTENSITY OF RESISTANCE TO PYRETHROIDS

Pyrethroid resistance intensity testing was done in eight sentinel districts. The resistance intensity of the populations tested was moderate for deltamethrin in 5 of 7 districts, for permethrin in 2 of 8 districts and for alpha-cypermethrin in 3 of 6 districts where the tests were conducted. High resistance intensity was observed in all sites (Figure 18).

Figure 17: Mortality Rate of *An. gambiae* s.l. against Deltamethrin 0.05%, Permethrin 0.75%, and Alpha-cypermethrin 0.05% after Pre-Exposure to Piperonyl Butoxide

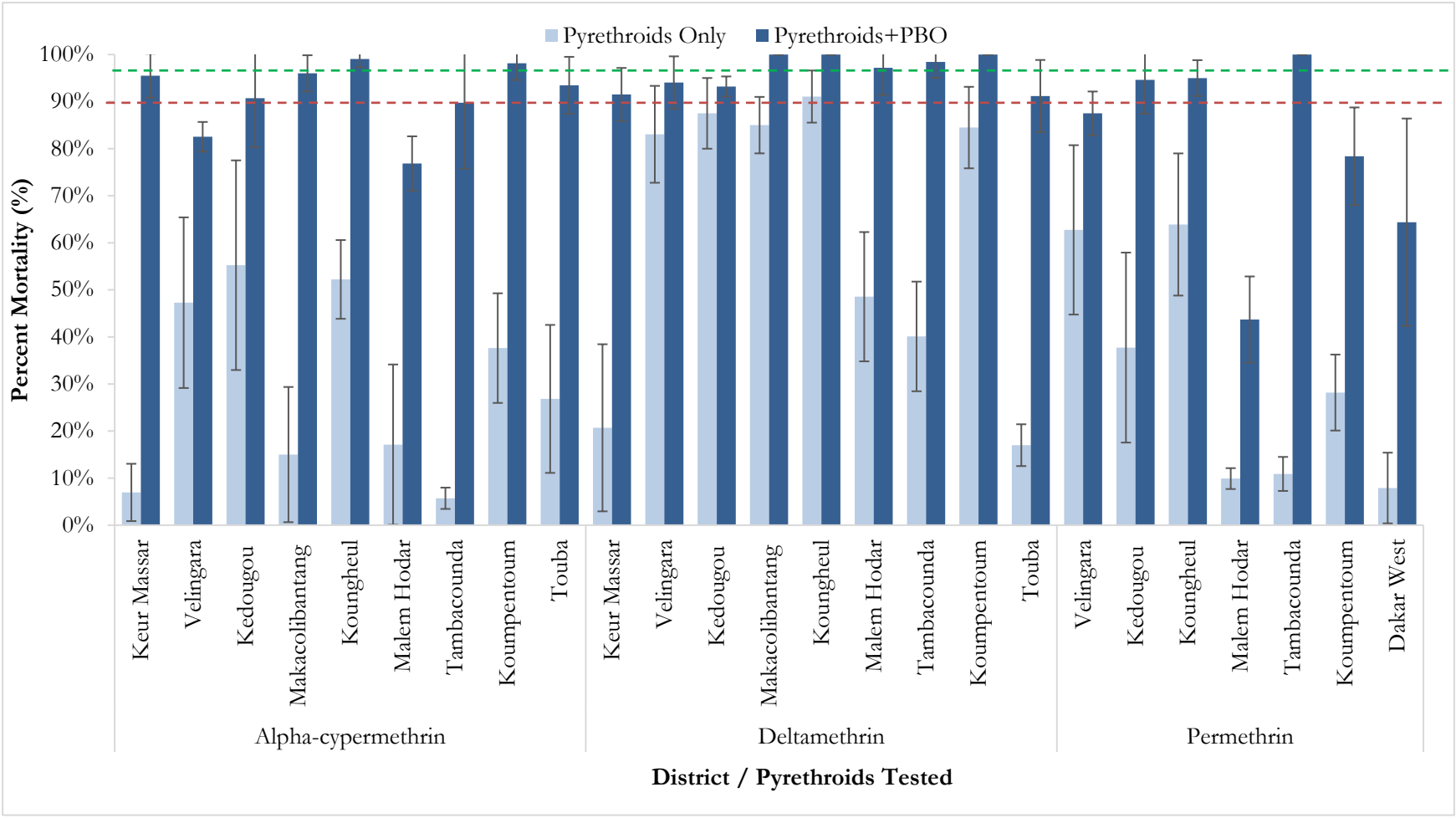
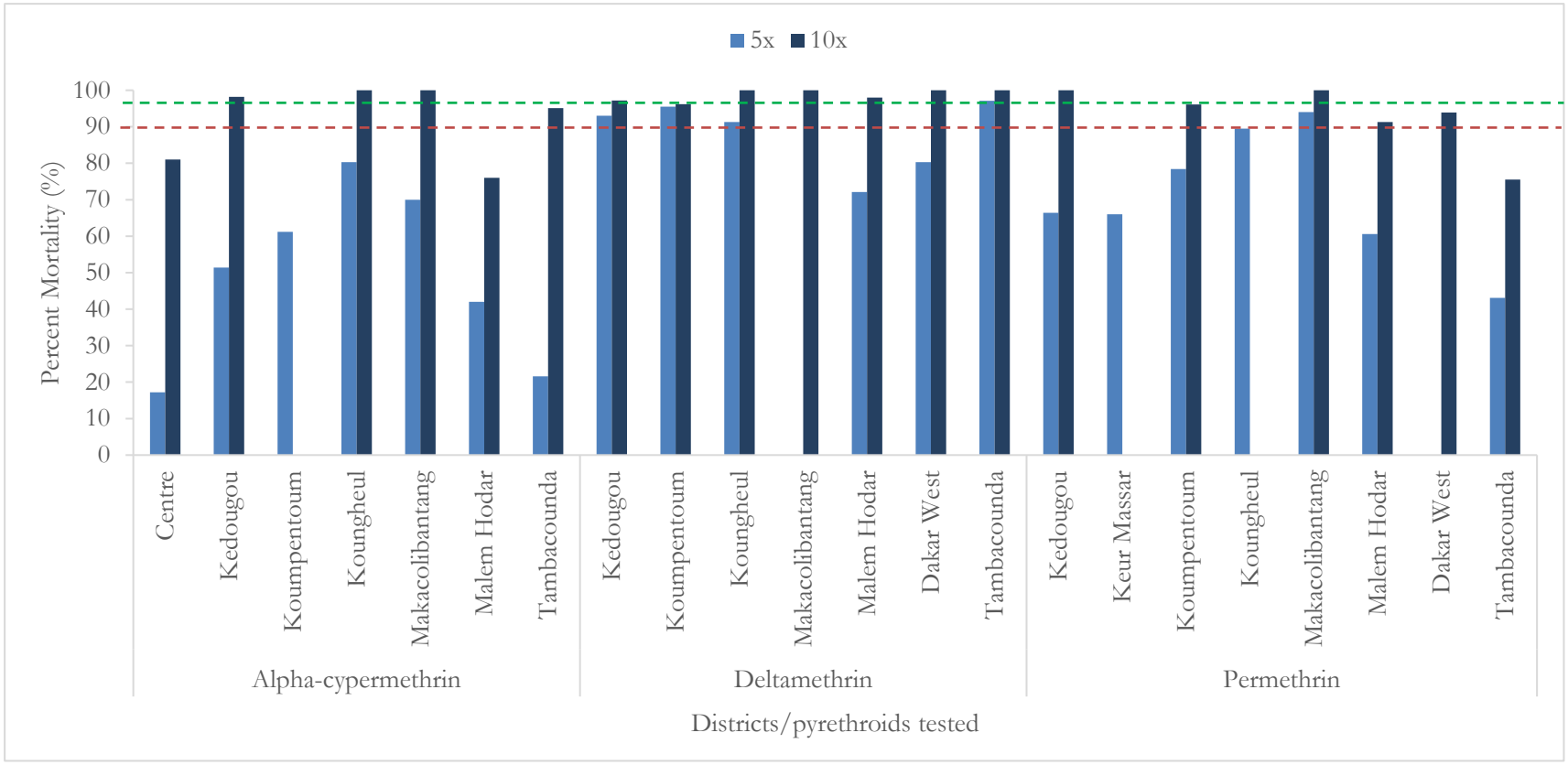


Figure 18: Resistance Intensity of *An. gambiae* s.l. by Sites Surveyed



3.3.3 SUSCEPTIBILITY OF *AN. GAMBIAE* S.L. TO PIRIMIPHOS-METHYL AND BENDIOCARB

An. gambiae s.l. populations were tested against bendiocarb and pirimiphos-methyl respectively in nine and seven districts, respectively. Susceptibility to pirimiphos-methyl and bendiocarb was recorded in *An. gambiae* s.l. populations in all districts monitored (Figures 20-21).

Figure 20: Susceptibility of *An. gambiae* s.l. to Bendiocarb

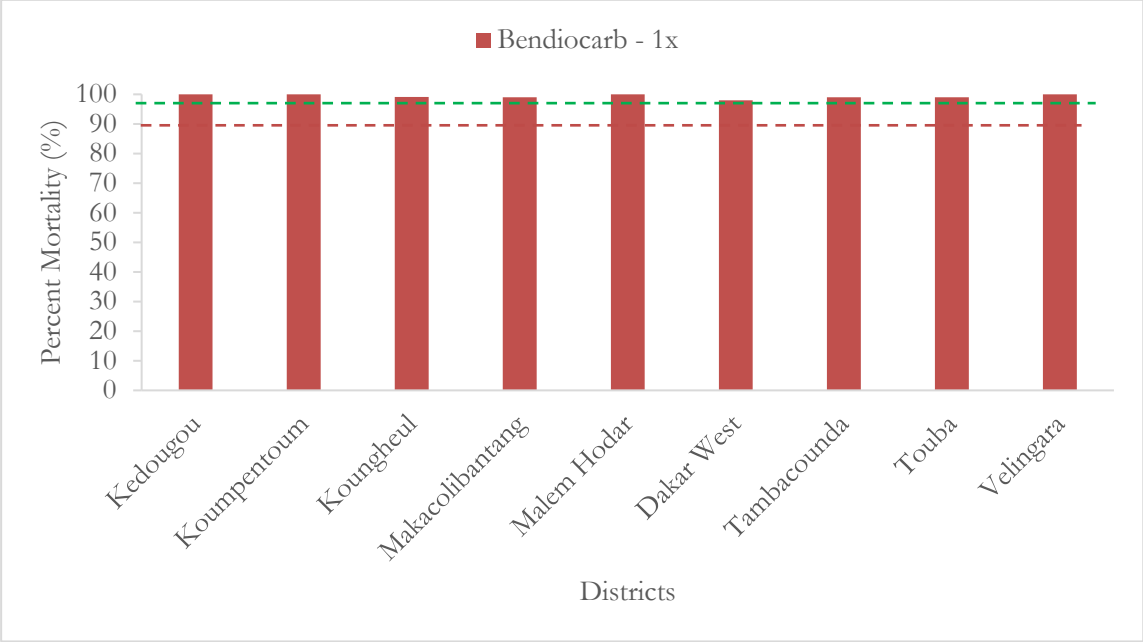
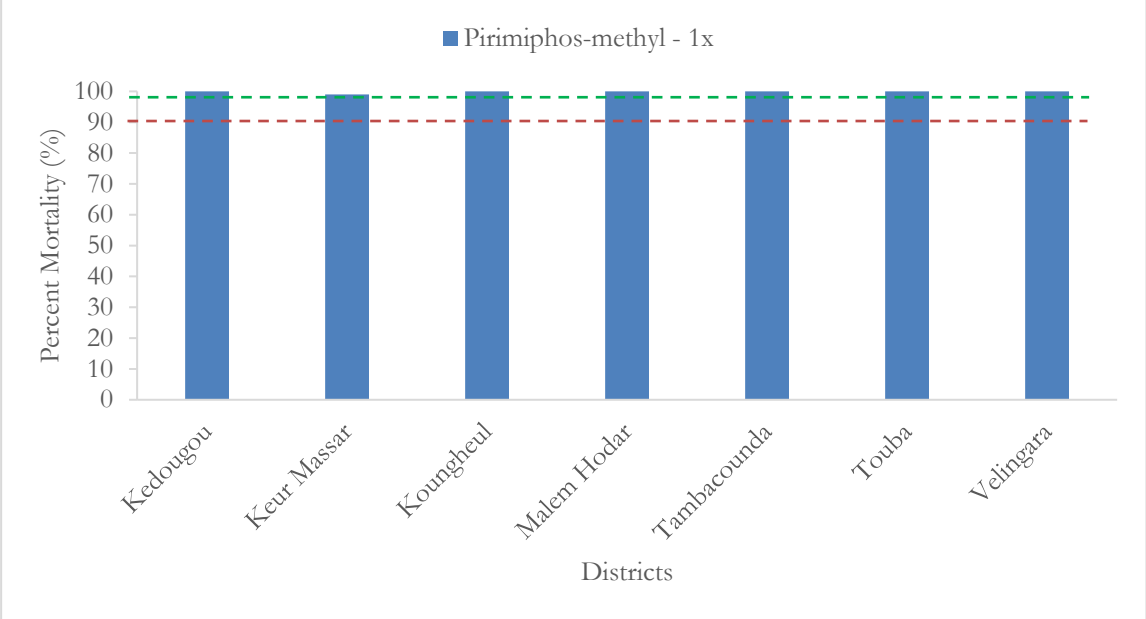


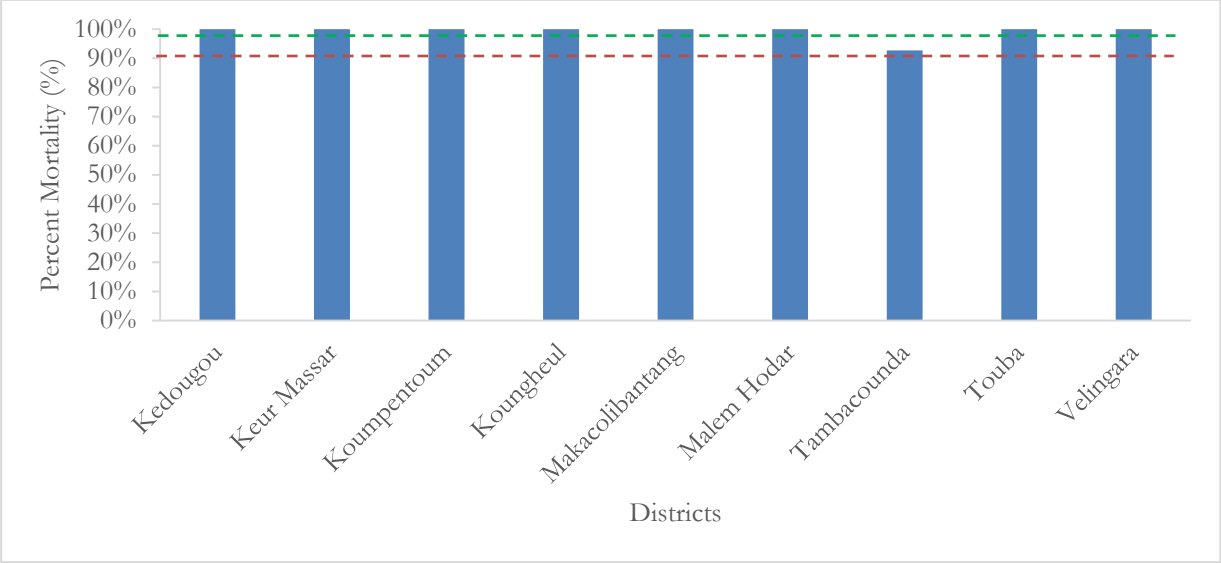
Figure 21: Susceptibility of *An. gambiae* s.l. to Pirimiphos-methyl



3.3.4 SUSCEPTIBILITY OF *AN. GAMBIAE* S.L. TO CLOTHIANIDIN

The susceptibility test with clothianidin was done in nine districts across the country (Figure 22). Susceptibility of *An. gambiae* s.l. to clothianidin 4 µg/bottle was recorded in all districts tested, with 100% mortality recorded at all districts, except Tambacounda (92.7%).

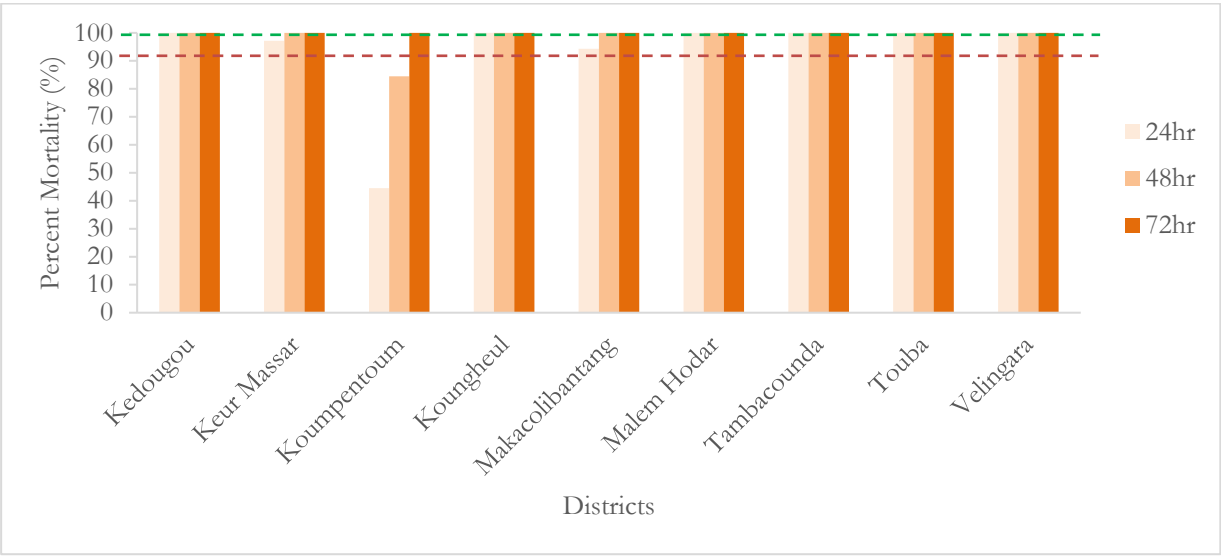
Figure 22: Susceptibility of *An. gambiae* s.l. to clothianidin 4 µg/bottle



3.3.5 SUSCEPTIBILITY OF *AN. GAMBIAE* S.L. TO CHLORFENAPYR

One hundred percent mortality of *An. gambiae* s. l. was recorded at 48 hours against chlorfenapyr 100 µg/bottle in 8 out of the 9 districts surveyed (Figure 23). It was only in Koumpentoum where 100% mortality was registered after 72h.

Figure 23: Susceptibility of *An. gambiae* s.l. to Chlorfenapyr 100µg/Bottle



3.4 Laboratory Analysis

3.4.1 Molecular Species Identification

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

A subsample of 2,795 female *An. gambiae* s.l. collected through HLC, PSCs, CDC-LT and Prokopack aspirator from April to November 2022 were analyzed by PCR for species determination. The results show that *An. arabiensis* (37.0%), *An. gambiae* (48.3%), *An. coluzzii* (9.5%), *hybrid An. gambiae / An. coluzzii* (0.1%) and *An. melas* (0.1%) were the species of the complex identified (Figure 24). 4.2% of non-amplified samples were recorded.

An. arabiensis was present in all districts and geographical zones (Figure 24). *An. melas* was only detected at Malem Hodar and Koungheul in the Sudano-Sahelian zone, which has semi-arid climates near the coastal inlet (Figure 25). *An. gambiae* is the prevalent species in the Sudano-Guinean zone, where it is found in sympatry with *An. arabiensis* and *An. coluzzii* (Figures 24 and 3; Annex B).

Figure 24: *An. gambiae* s.l. Species Composition and Distribution by Geographical Zone (April to December 2022)

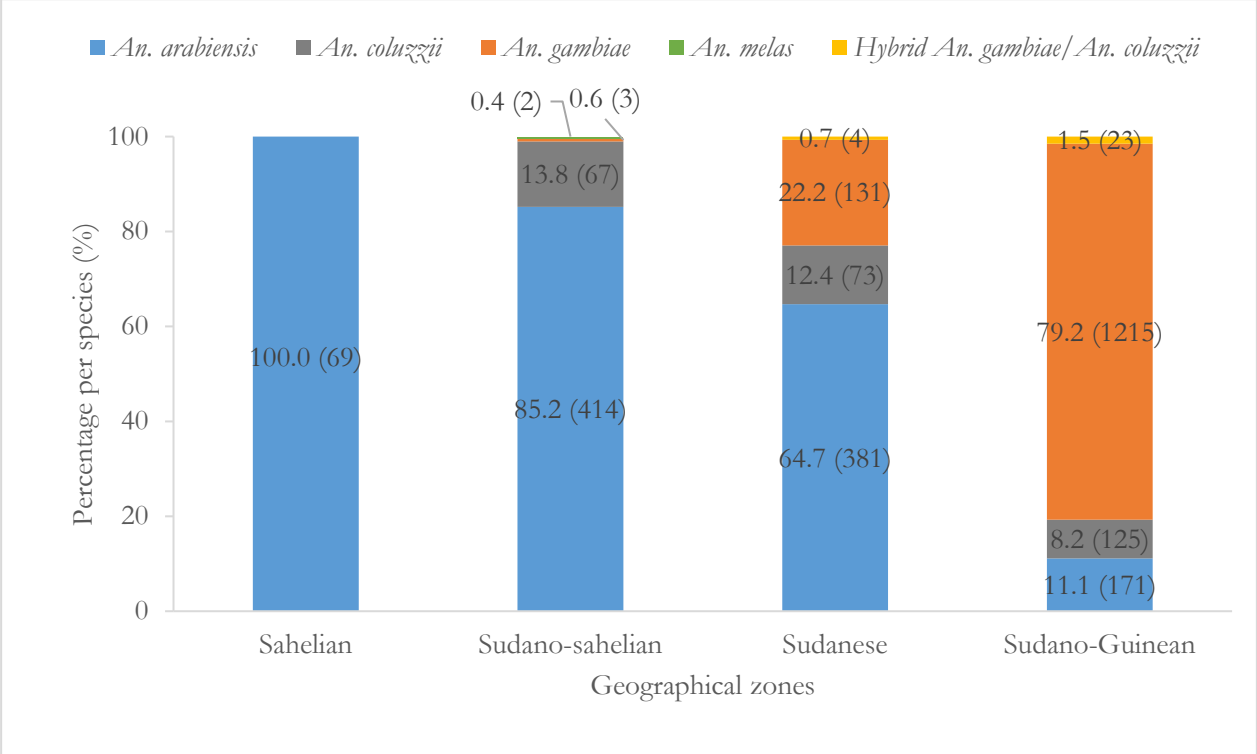
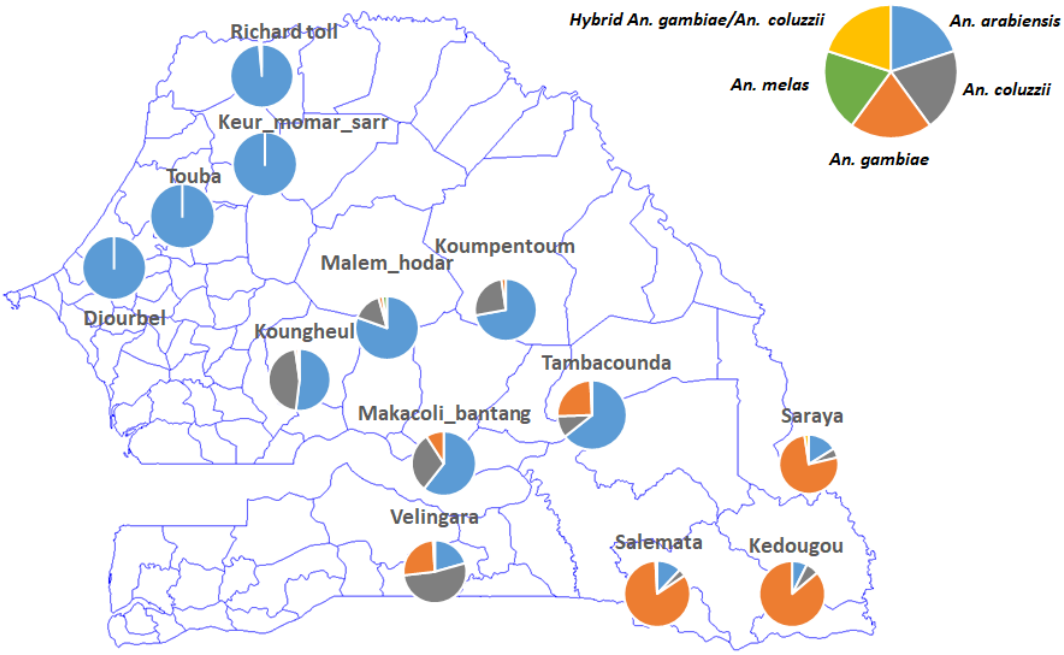


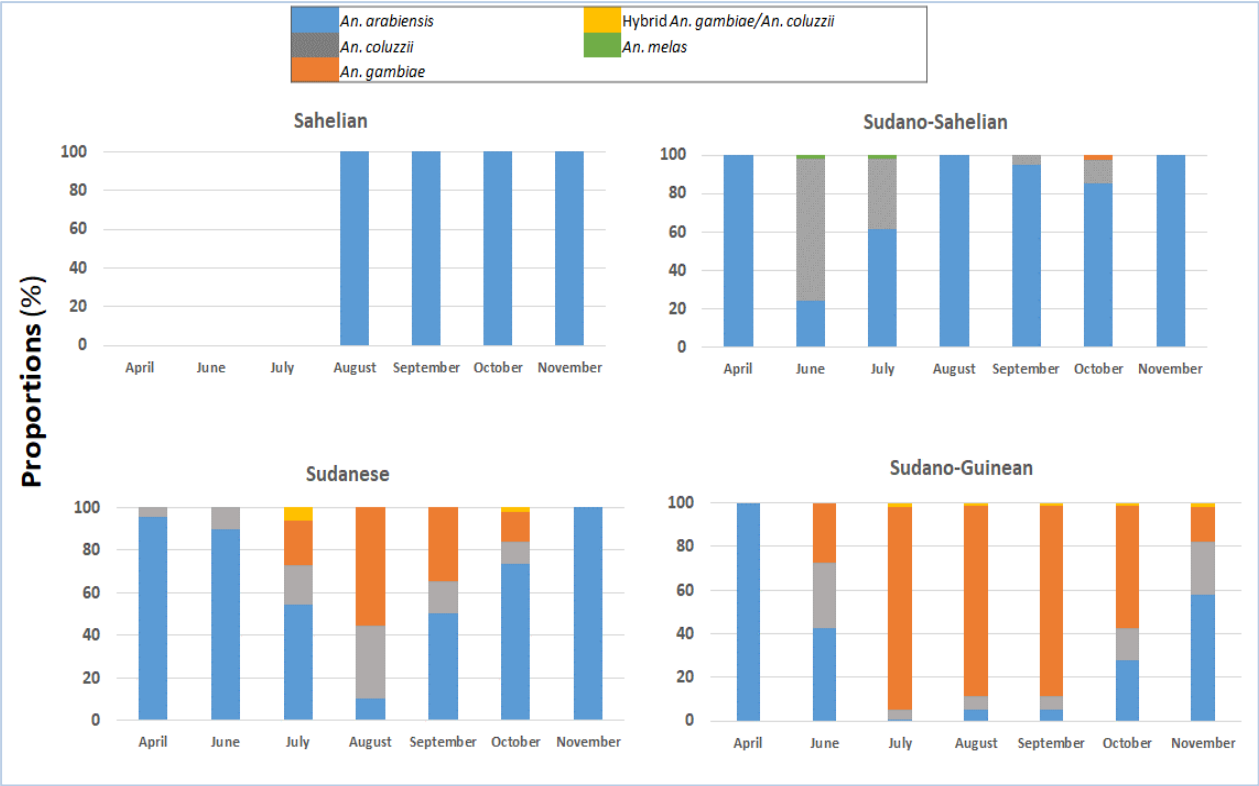
Figure 25: *An. gambiae* s.l. Species Composition and Distribution in Sentinel Sites (April to November 2022)



3.4.3 TEMPORAL VARIATION OF *AN. GAMBIAE* S.L.

Over the survey period, *An. arabiensis* was the most frequent species collected in the Sahelian, Sudano-Sahelian and Sudanese zones. In the Sudano-Guinean zone, *An. gambiae* was the most frequent member of the complex collected during the rainy season. (Figure 26; Annex C). Notably, in all the biogeographical zones where it was found, *An. coluzzii* appeared only during the rainy season, except in the Sahelian zone where it was absent at all times during the reporting period.

Figure 26: Monthly Variation in the Proportions of *An. gambiae* s.l. Species by Geographical Zones (January to November 2022)



3.4.4 SPECIES COMPOSITION OF *AN. FUNESTUS* S.L.

Molecular identification of species of *An. funestus* group showed that *An. funestus* s.s. was (n=51) the only species of the group present in all three geographical zones (Sudano-Sahelian: n=44, Sudanese: n=2 and Sudano-Guinean: n=5) where it was collected. All tested samples were successfully amplified.

3.4.5 ORIGIN OF BLOOD MEALS

3.4.5.1 AN. GAMBIAE S.L.

Analysis of 665 blood meals from *An. gambiae* s.l. fed females showed that 491 meals were identified and 174 were from unknown hosts. Of the 491 identified, 44% (215/491) were from humans, 49% (241/491) were from animal, 3 % (15/491) from Human+animal and 4% (20/491) from animal+animal In Sahelian and Sudano Sahelian zones, the anthropophilic rates (AR) were low, ranging from 2.8% in Richard Toll to 32% in Diourbel (Figures 27 and 28; Annex B4). The anthropophilic rates were higher in Sudanese (Makacolibantang: 67%) and Sudano-Guinean zones (Kédougou: 83%, Saraya/Salémata: 73% and Velingara: 86%). In PBO-ITN sites located in Sudanese area, AR (68%) was similar to Makacolibantang. The AR were comparable between IRS and control areas except for Tambacounda (Koussanar) where no female was fed on human and for Makacolibantang district which recorded the highest AR compared to all the sites monitored and Tambacounda (Koussanar) (Figure 27; Annex B4).

Figure 27: Blood Meal Sources of *An. gambiae* s.l. Females from IRS Districts and Associated Control

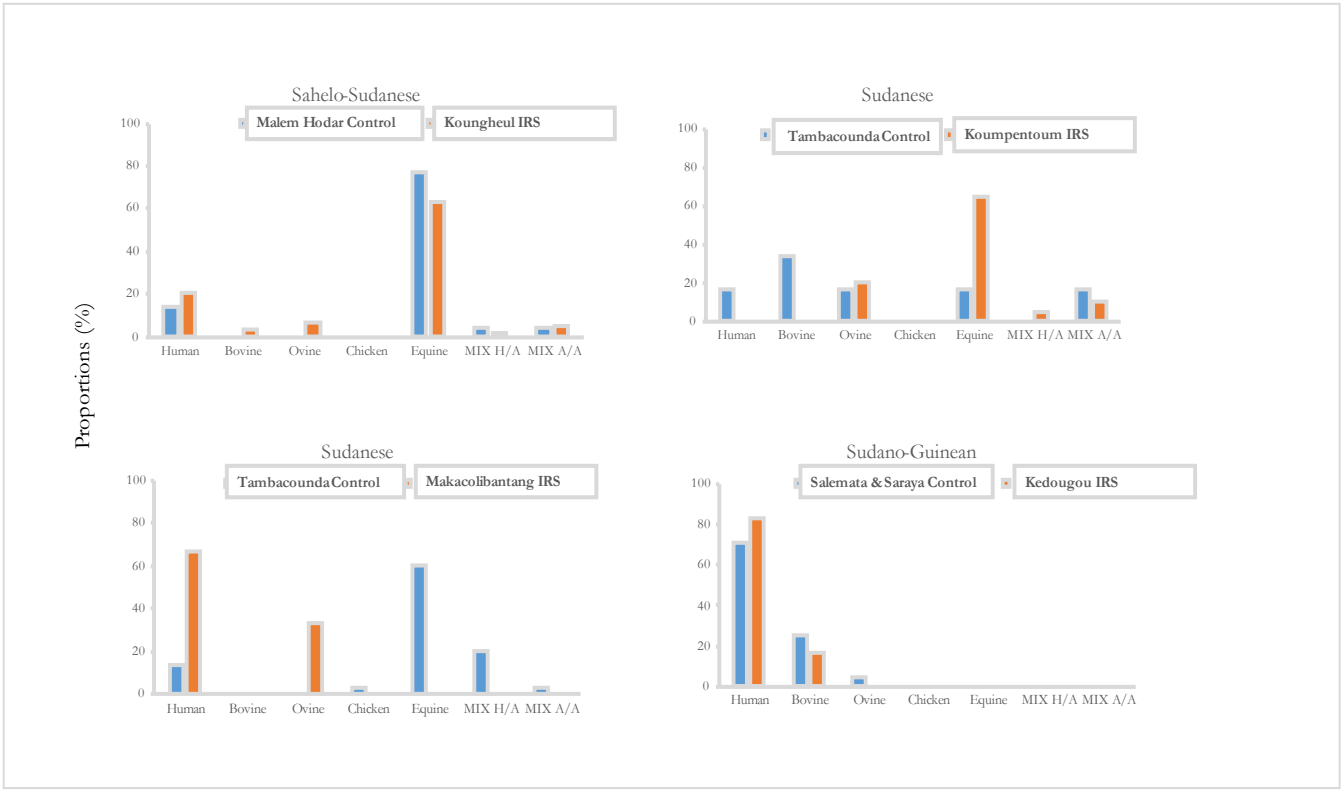
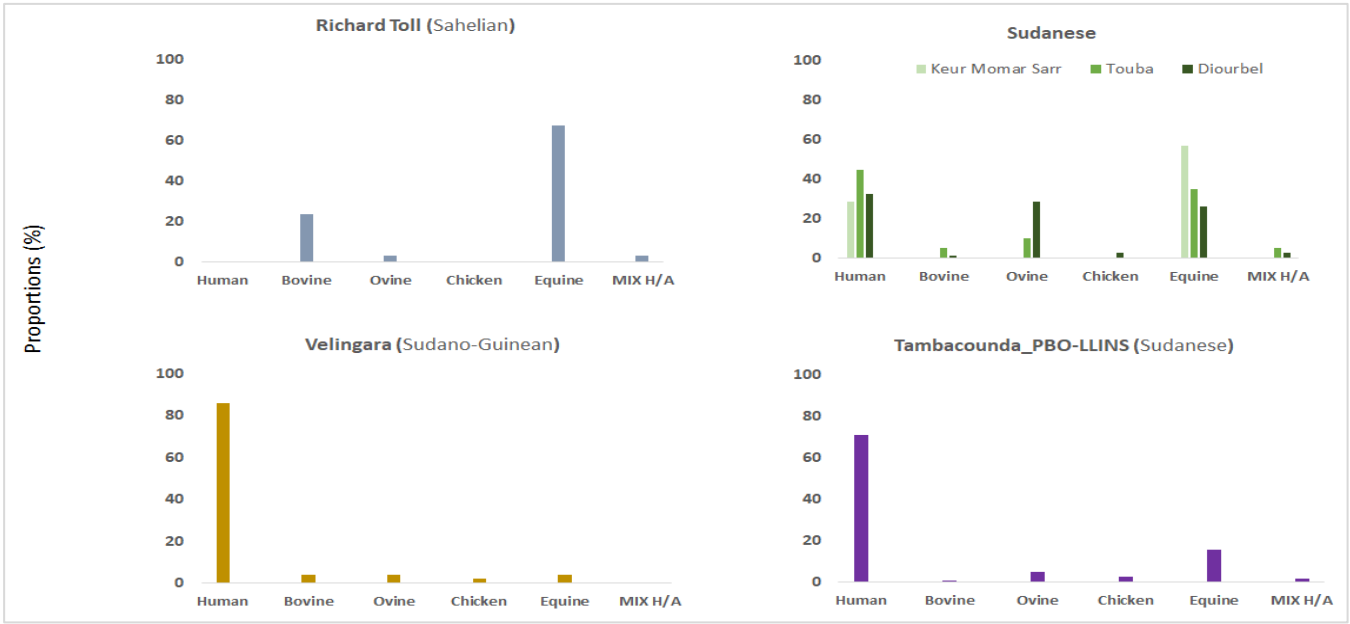


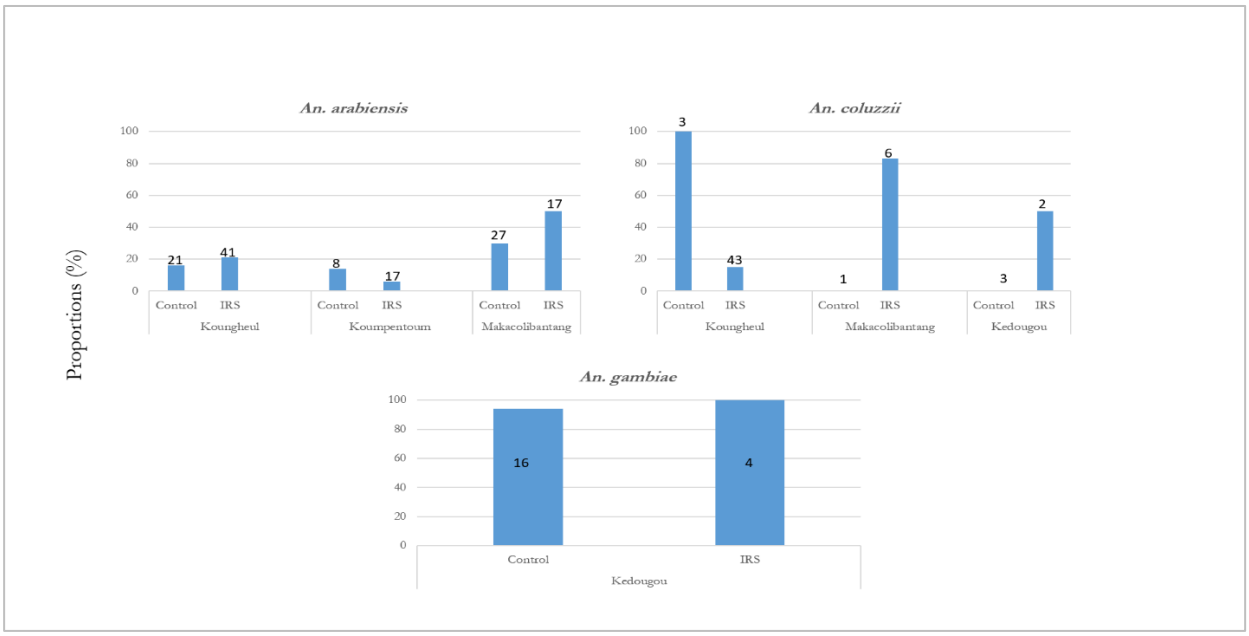
Figure 28: Blood Meal Sources of *An. gambiae* s.l. in Unsprayed Routine Vector Surveillance Districts



Overall, the anthropophilic rates were less than 50% for all the species of the *An. gambiae* complex within the Sahelian and Sahelo-Sudanese zones and Sudano-Sahelian zone of the country. However, in Malem Hodar (control site) and Diourbel (systematic vector surveillance site), only one *An. coluzzii* fed on human, was collected from each site. (Figures 22 & 23).

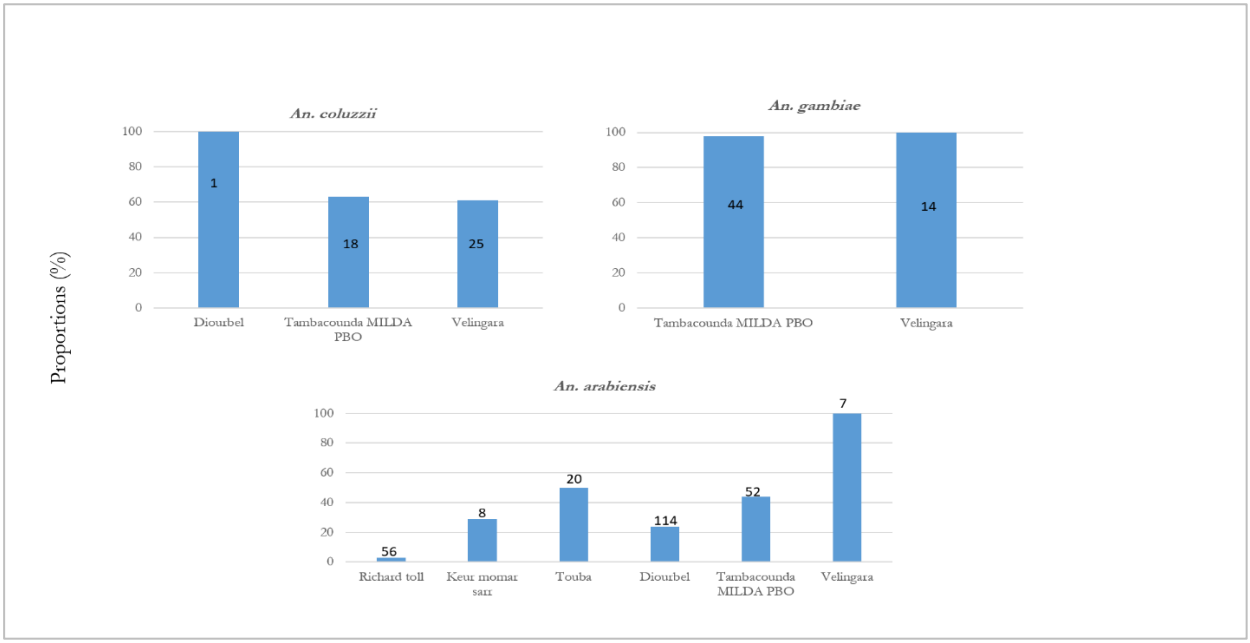
Among the members of the complex, *An. gambiae* is the most anthropophilic species with a high AR (≥ 75%) in the Sudano-Guinean zone both in the IRS/Control sites and in routine vector surveillance sites (Figure 29 & 30).

Figure 29: Anthropophilic Rates of *An. gambiae* s.l. Species in IRS Districts and Their Unsprayed Control



*Number on bars represent the number of mosquitoes tested per species

Figure 30: Anthropophilic Rates of *An. gambiae* s.l. Species in Unsprayed Routine Vector Surveillance Districts

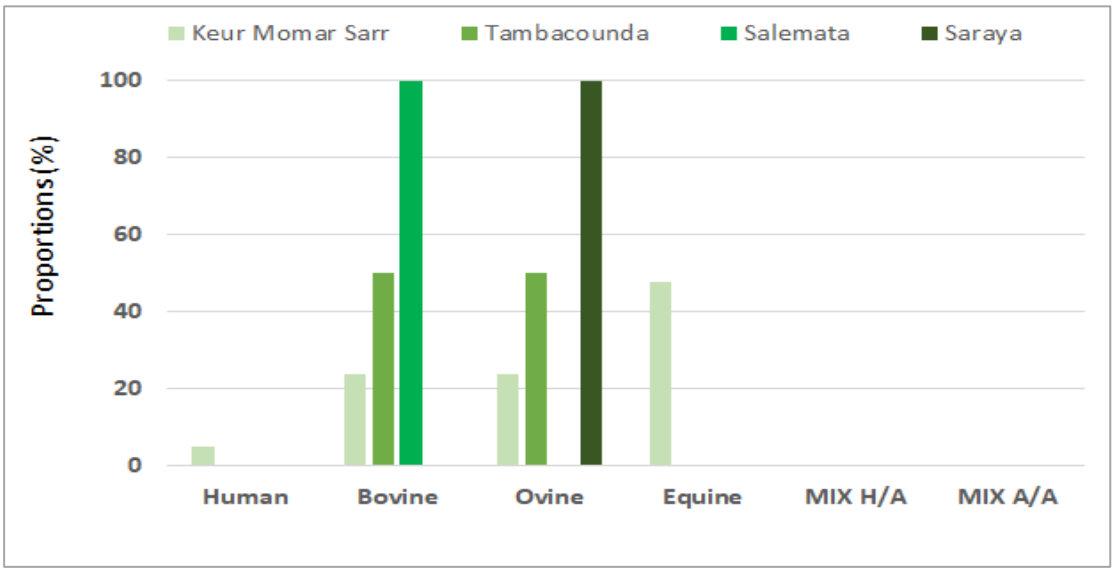


*Number on bars represent the number of mosquitoes tested per species

3.4.5.2 *AN. FUNESTUS* S.L.

The anthropophilic rate of *An. funestus* s.l. was low (4%: 1/25) in all districts where its presence has been noted. This vector was more zoophilic with a trophic preference for bovine and ovine in the Sudano-Guinean districts of Salémata (1/1) and Saraya (1/1) respectively zones and Equine in Sudano-Sahelian zone (Keur Momar Sarr: 10/21) (Figure 31; Annex B4).

Figure 31: Blood Meal Sources of *An. funestus* s.l.



3.4.6 *PLASMODIUM FALCIPARUM* INFECTION RATE

Tables 8 and 9 describe the results of circumsporozoite rate of *An. gambiae* and *An. funestus* collected by HLC. Only *An. gambiae* s.l. (n=1,832) was found positive for *P. falciparum* sporozoites and no infected females recorded from the 15 specimens of *An. funestus* tested during the study. The presence of infective females of *An. gambiae* s.l. was detected in the Sudanese and Sudano-Guinean zones. In IRS-districts, infected females were found only in Kédougou (sporozoite rate= 1.13%; 8/705). For unsprayed control districts, infected females were found in Tambacounda (Lycounda) (0.49%: 1/204) and Saraya/Salémata (2.58%: 21/815). The presence of infected females has also been recorded in the PBO-ITNs sites with a sporozoite rate of 0.65% (1/155). These infective females were collected in July (9/503), August (9/491), September (3/657), October (9/402) and November (1/227) (Annex B5). *An. nili* (1/138), collected in August, was found infected with *Plasmodium falciparum* in Salémata (Annex B6).

Table 8: *Plasmodium falciparum* Infection Rate in *An. gambiae* s.l. and *An. funestus* Group in IRS vs. Control Districts

Geographical areas	Districts	*An. gambiae* s.l.				*An. funestus* s.l.		
		T	P	CSI	P-value	T	P	CSI
Sudano-Sahelian	Koungheul (IRS)	47	0	0	NA	1	0	0
	Malem Hodar (Control)	11	0	0		0	0	0
Sudanese	Koumpentoum (IRS)	15	0	0	NA	0	0	0
	Koussanar (Control)	21	0	0		0	0	0
	Makacolibantang (IRS)	14	0	0	NA	0	0	0

	Lycounda (Control)	204	1	0.0049			0	0	0
Sudano-Guinean	Kédougou (IRS)	705	8	0.0113	P>0.05		2	0	0
	Saraya & Salémata (Control)	815	21	0.0258			12	0	0

T='Tested ; P= Positive ; CSI=Circumsporozoite index

Table 9: *Plasmodium falciparum* Infection Rate of *An. gambiae* s.l. and *An. funestus* Group in Unsprayed Vector Surveillance Districts

Geographical zones	DISTRICTS	*An. gambiae* s.l.			*An. funestus* s.l.		
		T	P	CSI	T	P	CSI
Sahelian	Richard Toll*	5	0	0	1	0	0
	Keur Momar Sarr*	1	0	0	21	0	0
Sudano-Sahelian	Touba*	24	0	0	0	0	0
	Diourbel*	114	0	0	0	0	0
Sudanese	Tambacounda (PBO-ITN site) *	155	1	0.0065	0	0	0
Sudano-Guinean	Velingara*	44	0	0	0	0	0

T=Tested; P= Positive; CSI=Circumsporozoite index, * Community-based mosquito surveillance

Among the species of the *An. gambiae* s.l. tested (1785), *An. coluzzii*/*An. gambiae* hybrid (5%: 1/22) recorded the highest sporozoite rates. It is followed by *An. gambiae* (2%: 21/1234) and *An. coluzzii* (1%: 1/119). No *P. falciparum*–infected specimen was found among the *An. arabiensis* tested (Table 10; Annex B7).

Table 10: *Plasmodium falciparum* Infection Rate of the *An. gambiae* s.l. Species Collected by HLC in the IRS District and Their Control (April to December 2022)

Geographical zones	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)	25	0	0	2	0	0	16	0	0	0	0	0
	Control	8	0	0	0	0	0	2	0	0	0	0	0
Sudanese	Koumpentoum (IRS)	8	0	0	1	0	0	3	0	0	0	0	0
	Control	14	0	0	2	0	0	2	0	0	0	0	0
	Makacolibantang (IRS)	9	0	0	1	0	0	2	0	0	0	0	0
	Control	151	0	0	13	0	**0**	13	0	0	1	0	0
Sudano-Guinean	Kédougou (IRS)	46	0	0	568	6	**0.011**	40	1	**0.025**	10	1	**0.1**
	Control (Saraya & Salemata)	89	0	0	590	14	**0.024**	26	0	0	10	0	0
Sudanese	Tambacounda (PBO-LLIN-site)	60	0	0	57	1	0.018	15	0	0	1	0	0

Key: T=tested, P=positive, CSI=circumsporozoite index.

3.4.7 ENTOMOLOGICAL INOCULATION RATE (EIR)

The highest EIR was recorded in the Sudano-Guinean zone, particularly in the districts of Kédougou (0.093 ib/p/n) and Salémata (0.307 ib/p/n), where most of transmission is ensured by *An. gambiae*, both inside and outside dwellings (Tables 11 & 12; Annex B8).

Table 11: *Plasmodium falciparum* Infection Rate of *An. gambiae* s.l. and Entomological Inoculation Rate Collected by HLC in the Surveyed Sites

Geographical zones	District	*An. gambiae* s.l.			*An. funestus* s.l.		
		HBR	**CSI**	**EIR**	**HBR**	**CSI**	**EIR**
Sudano-Sahelian	Koungheul (IRS)	0.298	0.00	0.00	0.006	0.00	0.00
	Malem Hodar (Control)	0.065	0.00	0.00	0.00	0.00	0.00
Sudanese	Koumpentoum (IRS)	0.089	0.00	0.00	0.00	0.00	0.00
	Koussanar (Control)	0.321	0.00	0.00	0.00	0.00	0.00
	Makacolibantang (IRS)	0.089	0.00	0.00	0.00	0.00	0.00
	Lycounda (Control)	2.548	0.0049	**0.012**	0.00	0.00	0.00
	Kédougou (IRS)	8.22	0.0113	**0.093**	0.012	0.00	0.00
	Tambacounda (PBO-ITNs)	0.565	0.0065	**0.004**	0.00	0.00	0.00
Sudano-Guinean	Saraya & Salemata (Control)	6.667	0.0258	**0.172**	0.077	0.00	0.00

Key: HBR=human biting rate, CSI=circumsporozoite index, EIR=entomological inoculation rate.

Table 12: *Plasmodium falciparum* Infection Rate and Entomological Inoculation Rate of *An. gambiae* s.l. Species in the Surveyed Sites (April to December 2022)

Geographical zones	Districts	*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 (IRS)	0.149	0.00	0.00	0.012	0.00	0.00	0.095	0.00	0.00	0.00	0.00	0.00
	Malem Hodar (Control)	0.048	0.00	0.00	0.000	0.00	0.00	0.012	0.00	0.00	0.00	0.00	0.00
Sudanese	Koumpentoum (IRS)	0.048	0.00	0.00	0.006	0.00	0.00	0.018	0.00	0.00	0.00	0.00	0.00
	Koussanar (Control)	0.167	0.00	0.00	0.024	0.00	0.00	0.024	0.00	0.00	0.00	0.00	0.00
	Makacolibantang (IRS)	0.054	0.00	0.00	0.006	0.00	0.00	0.012	0.00	0.00	0.00	0.00	0.00
	Lycounda (Control)	1.798	0.00	0.00	0.155	0.00	0.00	0.155	0.00	0.00	0.012	0.00	0.00
	Kédougou (IRS)	0.274	0.00	0.00	3.399	0.011	**0.036**	0.238	0.025	**0.006**	0.071	0.100	**0.007**
Sudano-Guinean	Tambacounda (PBO-ITNs)	0.179	0.00	0.00	0.170	0.018	**0.003**	0.045	0.00	0.00	0.003	0.00	0.00
	Saraya & Salemata (Control)	0.53	0.00	0.00	3.53	0.024	**0.084**	0.155	0.00	0.00	0.06	0.00	0.00

Key: HBR=human biting rate, CSI=circumsporozoite index, EIR=entomological inoculation rate.

3.4.8 *KDR*-EAST (L1014S) AND *KDR*-WEST (L1014F) MUTATIONS

3.4.8.1 GENOTYPES AND ALLELIC FREQUENCIES

Genotyping results revealed the presence of both mutations in all the districts (Figure 32) except in Makacolibantang where *Kdr*-east was not detected. The frequency of *Kdr*-west was higher in Kédougou (56.7%) districts but lower in Koungheul (7.1%). For *Kdr*-east, higher frequency was observed in Touba (59.1%), Malem

Hodar (46.7%) and Dakar (46.7%) than the other sites (Koungheul (3.6%)) (Figure 25 & Table 14). This confirms the higher *kdr* west frequency across the country than the east mutation. However, sites like Touba, Malem Hodar and Dakar will need close follow up and confirmation of the *kdr* east mutation, which is surprisingly high at these sites.

The co-occurrence of both mutations (*Kdr*-west and *Kdr*-east) in the same individual, resulting in a resistant heterozygous genotype RwRe, was also noted in all districts except in Tambacounda, Vélingara and Kédougou (Table 20). The highest frequencies of this co-occurrence were recorded in Rufisque, Pikine and Diourbel, ranging from 18% in Pikine to 39% in Rufisque.

Figure 32: Distribution of Allelic Frequencies of *Kdr*-west (L1014F) and *Kdr*-east (L1014S) Mutations in Sentinel Districts

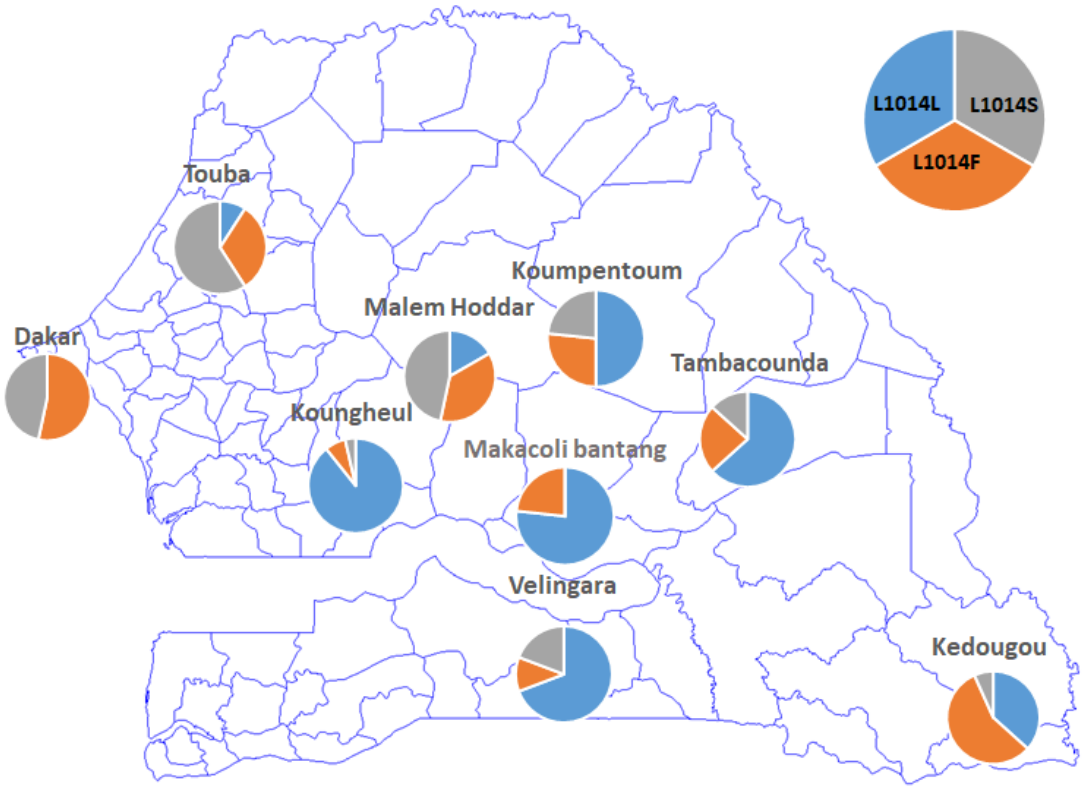


Table 13: Genotypic and Allelic Frequencies of *Kdr*-west and *Kdr*-east Mutations in *An. gambiae* s.l. by District

Districts	Total	Genotype (%)						Total	Allele (%)		
		SS	SRw	SRe	RwRw	RwRe	ReRe		S	Rw	Re
Dakar	15	0 (0.0)	0 (0.0)	0 (0.0)	5 (33.3)	6 (40.0)	4 (26.7)	30	0 (0.0)	16 (53.3)	14 (46.7)
Touba	11	0 (0.0)	1 (9.1)	1 (9.1)	1 (9.1)	4 (36.4)	4 (36.4)	22	2 (9.1)	7 (31.8)	13 (59.1)
Koungheul	14	12 (85.7)	1 (7.1)	0 (0.0)	0 (0.0)	1 (7.1)	0 (0.0)	28	25 (89.3)	2 (7.1)	1 (3.6)
Malem Hodar	15	1 (6.7)	0 (0.0)	3 (20.0)	3 (20.0)	5 (33.3)	3 (20.0)	30	5 (16.7)	11 (36.7)	14 (46.7)
Koumpentoum	15	4 (26.7)	4 (26.7)	3 (20.0)	1 (6.7)	2 (13.3)	1 (6.7)	30	15 (50.0)	8 (26.7)	7 (23.3)
Tambacounda	15	8 (53.3)	2 (13.3)	1 (6.7)	2 (13.3)	1 (6.7)	1 (6.7)	30	19 (63.3)	7 (23.3)	4 (13.3)
Makacolibantang	15	9 (60.0)	5 (33.3)	0 (0.0)	1 (6.7)	0 (0.0)	0 (0.0)	30	23 (76.7)	7 (23.3)	0 (0.0)
Kédougou	15	2 (13.3)	5 (33.3)	2 (13.3)	6 (40.0)	0 (0.0)	0 (0.0)	30	11 (36.7)	17 (56.7)	2 (6.7)
Velingara	13	7 (53.9)	2 (15.4)	2 (15.4)	0 (0.0)	1 (7.7)	1 (7.7)	26	18 (69.2)	3 (11.5)	5 (19.2)

3.4.8.2 GENOTYPIC FREQUENCY OF *KDR* MUTATIONS ACCORDING TO THE MOSQUITO PHENOTYPIC STATUS (ALIVE VS. DEAD)

The frequency of *Kdr*-west was higher in the live mosquitoes in all districts except Koungheul where it was higher in the dead (dead: 10%. alive: 0%). However, no difference was observed between the frequencies of *Kdr*-west in the two phenotypes (alive: 50%, dead: 55.6%) in Dakar (Table 15). *Kdr*-east mutation frequencies were relatively higher in live mosquitoes than dead in Koumpentoum, Kédougou, Vélingara and Tambacounda. But the frequency of the mutation was higher in the dead (alive: 25%; dead: 54.6%), in Malem Hodar (Table 15).

Table 14: 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 (%)							Allele (%)			
		N	SS	SRw	SRe	RwRw	RwRe	ReRe	N	S	Rw	Re
Dakar	Dead	9	0 (0.0)	0 (0.0)	0 (0.0)	4 (44.4)	2 (22.2)	3 (33.3)	18	0 (0)	10 (55.6)	8 (44.4)
	Alive	6	0 (0.0)	0 (0.0)	0 (0.0)	1 (16.7)	4 (66.7)	1 (16.7)	12	0 (0)	6 (50.0)	6 (50)
Touba	Dead	6	0 (0.0)	1 (16.7)	1 (16.7)	1 (16.7)	0 (0.0)	3 (50.0)	12	2 (16.7)	3 (25.0)	7 (58.3)
	Alive	5	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	4 (80.0)	1 (20.0)	10	0 (0.0)	4 (40.0)	6 (60.0)
Koungheul	Dead	10	8 (80.0)	1 (10.0)	0 (0.0)	0 (0.0)	1 (10)	0 (0.0)	20	17 (85)	2 (10)	1 (5.0)

Districts	Status	Genotype (%)							Allele (%)			
		N	SS	SRw	SRe	RwRw	RwRe	ReRe	N	S	Rw	Re
Malem Hodar	Alive	4	4 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0)	0 (0.0)	8	8 (100)	0 (0)	0 (0)
	Dead	11	1 (9.1)	0 (0.0)	3 (27.3)	1 (9.1)	3 (27.3)	3 (27.3)	22	5 (22.7)	5 (22.7)	12 (54.6)
Koumpentoum	Alive	4	0 (0.0)	0 (0.0)	0 (0.0)	2 (50)	2 (50)	0 (0.0)	8	0 (0.0)	6 (75.0)	2 (25.0)
	Dead	11	3 (27.3)	2 (18.2)	3 (27.3)	1 (9.1)	1 (9,09)	1 (9.1)	22	11 (50.0)	5 (22.7)	6 (27.3)
Tambacounda	Alive	4	1 (25)	2 (50.0)	0 (0.0)	0 (0)	1 (25)	0 (0.0)	8	4 (50)	3 (37.5)	1 (12.5)
	Dead	11	8 (72.7)	1 (9.1)	1 (9.1)	1 (9.1)	0 (0)	0 (0.0)	22	18 (81.8)	3 (13.6)	1 (4.5)
Maka colibantang	Alive	4	0 (0.0)	1 (25.0)	0 (0.0)	1 (25.0)	1 (25.0)	1 (25.0)	8	1 (12.5)	4 (50.0)	3 (37.5)
	Dead	11	8 (72.7)	3 (27.3)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	22	19 (86.4)	3 (13.6)	0 (0.0)
Kédougou	Alive	4	1 (25.0)	2 (50.0)	0 (0.0)	1 (25.0)	0 (0.0)	0 (0.0)	8	4 (50.0)	4 (50.0)	0 (0.0)
	Dead	12	2 (16.7)	4 (33.3)	1 (8.3)	5 (41.7)	0 (0.0)	0 (0.0)	24	9 (37.5)	14 (58.3)	1 (4.2)
Vélingara	Alive	3	0 (0.0)	1 (33.3)	1 (33.3)	1 (33.3)	0 (0.0)	0 (0.0)	6	2 (33.3)	3 (50.0)	1 (16.7)
	Dead	11	7 (63.6)	1 (9.1)	2 (18.2)	0 (0.0)	1 (9.1)	0 (0.0)	22	17 (77.3)	2 (9.1)	3 (13.6)
	Alive	2	0 (0.0)	1 (50.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (50.0)	4	1 (25.0)	1 (25.0)	2 (50.0)

Note: few unamplified samples were recorded in Touba (4), Koungheul (1) and Velingara (2).

3.4.8.3 GENOTYPIC FREQUENCY OF *KDR* MUTATIONS BY SPECIES

Genotyping of the species of the *Anopheles gambiae* complex (Table 16) showed the presence of *Kdr*-west mutations in the three identified species *An. arabiensis, An. coluzzii* and *An. gambiae*). But the *Kdr*-east mutation was not found in *An. coluzzii.*

Table 15: Genotypic Prevalence of the *Kdr*-west and *Kdr*-east Mutations by Species (*An. coluzzii* and *An. arabiensis*)

Districts	Species	Genotype (%)							Allele (%)			
		N	SS	SRw	SRe	RwRw	RwRe	ReRe	N	S	Rw	Re
Dakar	*An. arabiensis*	15	0 (0.0)	0 (0.0)	0 (0.0)	5 (33.3)	6 (40.0)	4 (26.7)	30	0 (0.0)	16 (53.3)	14 (46.7)
Touba	*An. arabiensis*	11	0 (0.0)	1 (9.1)	1 (9.1)	1 (9.1)	4 (36.4)	4(36.4)	22	2 (9.1)	7 (31.8)	13 (59.1)
Koungheul	*An. arabiensis*	14	12 (85.7)	1 (7.1)	0 (0.0)	0 (0.0)	1(7.1)	0 (0.0)	28	25 (89.3)	2 (7.1)	1 (3.6)
Malem Hodar	*An. arabiensis*	15	1 (6.7)	0 (0.0)	3 (20.0)	3 (20.0)	5 (33.3)	3 (20.0)	30	5 (16.7)	11 (36.7)	14 (46.7)
Koumpentoum	*An. coluzzii*	2	0 (0.0)	2 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	4	2 (50.0)	2 (50.0)	0 (0.0)

Districts	Species	Genotype (%)								Allele (%)			
		N	SS	SRw	SRe	RwRw	RwRe	ReRe		N	S	Rw	Re
	An. arabiensis	13	4 (30.8)	2 (15.4)	3 (23.1)	1 (7.7)	2(15.4)	1(7.7)		26	13 (50.0)	6 (23.1)	7 (26.9)
Tambacounda	*An. gambiae*	3	1 (33.3)	0 (0.0)	0 (0.0)	2 (66.7)	0 (0.0)	0 (0.0)		6	2 (33.3)	4 (66.7)	0 (0.0)
	An. arabiensis	12	7 (58.3)	2 (16.7)	1 (8.3)	0 (0.0)	1 (8.3)	1 (8.3)		24	17 (70.8)	3 (12.5)	4 (16.7)
Makacolibantang	*An. arabiensis*	15	9 (60.0)	5 (33.3)	0 (0.0)	1 (6.7)	0 (0.0)	0 (0.0)		30	23 (76.7)	7 (23.3)	0 (0.0)
Kédougou	*An. coluzzii*	3	0 (0.0)	2 (66.7)	0 (0.0)	1 (33.3)	0 (0.0)	0 (0.0)		6	2 (33.3)	4 (66.7)	0 (0.0)
	An. gambiae	6	0 (0.0)	1 (16.7)	0 (0.0)	5 (83.3)	0 (0.0)	0 (0.0)		12	1 (8.3)	11 (91.7)	0 (0.0)
	An. arabiensis	6	2 (33.3)	2 (33.3)	2 (33.3)	0 (0.0)	0 (0.0)	0 (0.0)		12	8 (66.7)	2 (16.7)	2 (16.7)
Velingara	*An. coluzzii*	1	1 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)		2	2 (100.0)	0 (0.0)	0 (0.0)
	An. arabiensis	12	6 (50.0)	2 (16.7)	2 (16.7)	0 (0.0)	1 (8.3)	1 (8.3)		24	16 (66.7)	3 (12.5)	5 (20.8)

Key: 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 allele.

3.4.9 ACE-1 MUTATION

The results of the *Ace-1* mutation showed the presence of the resistant allele only in the districts of Tambacounda (0.03%) and Kédougou (0.03%) with very low frequencies in the survivors of the *An. gambiae* s.l. (Table 16).

Table 16: Genotype Frequencies for the *Ace 1* Mutation in *An. gambiae* s.l.

		Genotypes (%)					Allele (%)	
District	**N**	**SS**	**RS**	**RR**		**N**	**S**	**R**
Dakar	15	15 (100)	0 (0)	0 (0)		30	30 (100)	0 (0)
Touba	14	14 (100)	0 (0)	0 (0)		28	28(100)	0 (0)
Koungheul	15	15 (100)	0 (0)	0 (0)		30	30 (100)	0 (0)
Malem Hodar	15	15 (100)	0 (0)	0 (0)		30	30 (100)	0 (0)
Koumpentoum	15	15 (100)	0 (0)	0 (0)		30	30 (100)	0 (0)
Tambacounda	15	14 (93.3)	01(6.67))	0 (0)		30	29 (96.67)	01(3.33)
Makacolibantang	15	15 (100)	0 (0)	0 (0)		30	30 (100)	0 (0)
Kedougou	15	14 (93.3)	01(6.67))	0 (0)		30	29 (96.67)	01(3.33)
Velingara	15	15 (100)	0 (0)	0 (0)		30	30 (100)	0 (0)

3.5 WALL BIOASSAYS

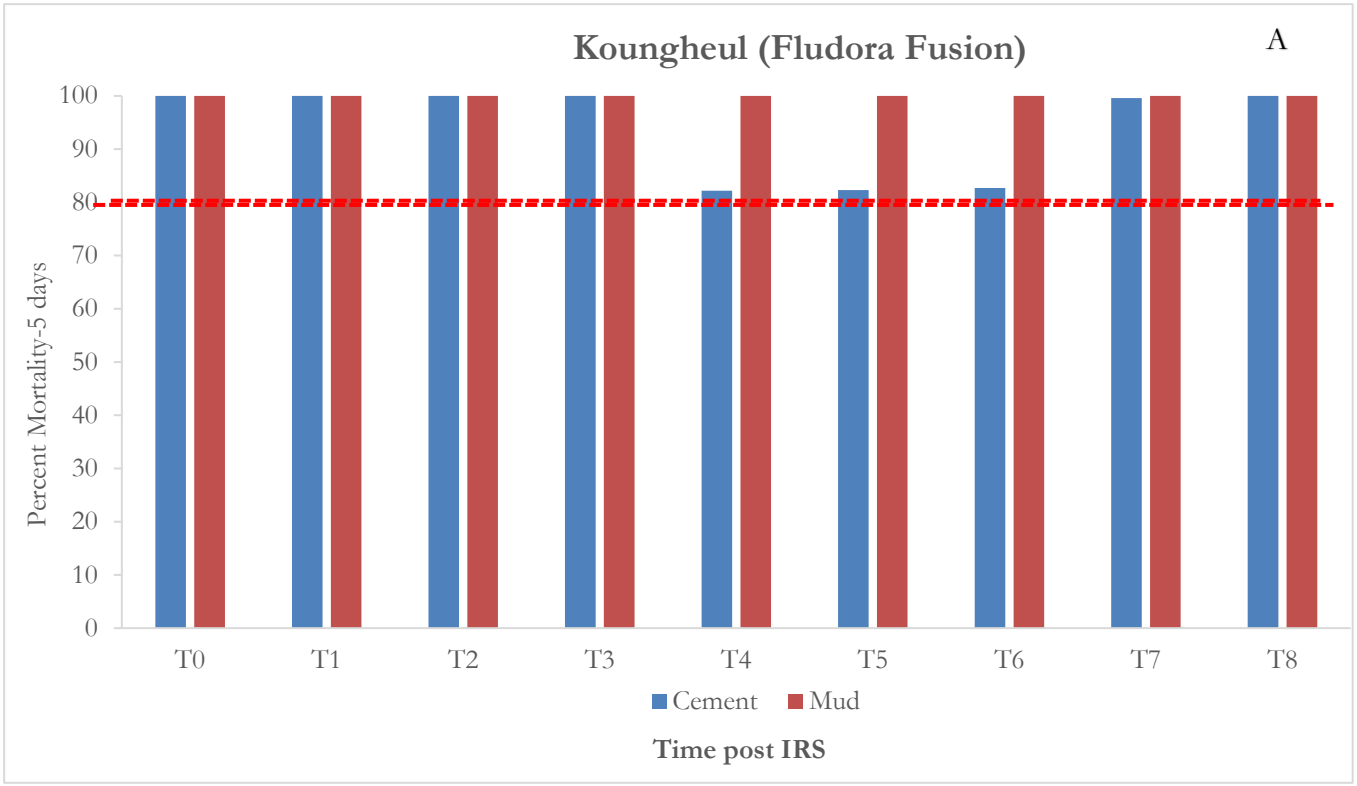
3.5.1 IRS SPRAY QUALITY ASSURANCE

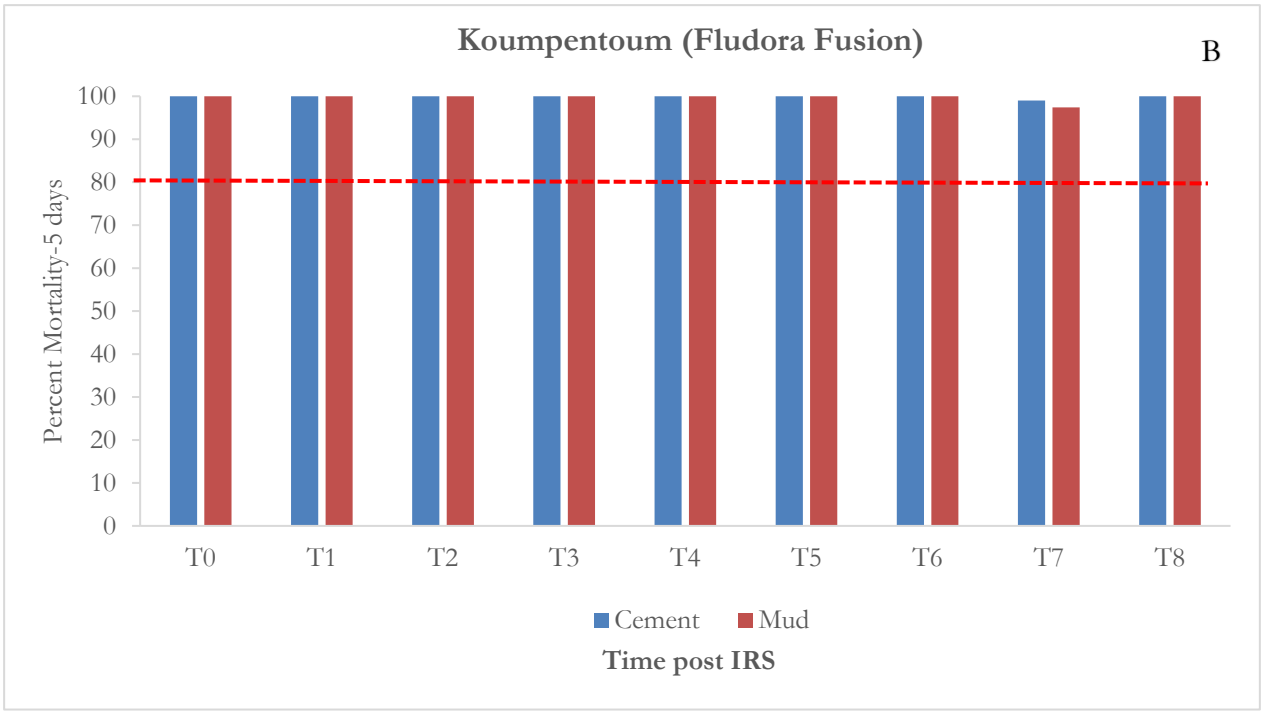
A hundred percent mortality (100%) was recorded against susceptible laboratory-reared *An. coluzzii* on all tested cement and mud walls in all IRS sites indicating good quality of the spraying.

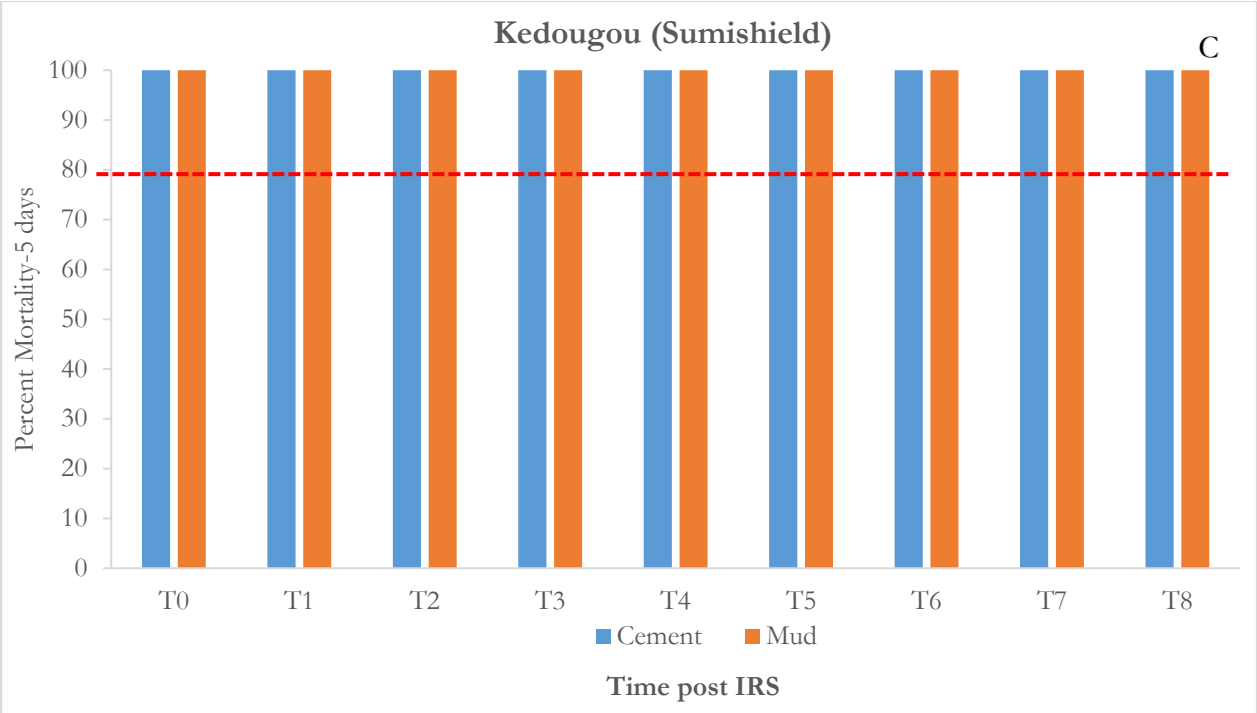
3.5.2 MONTHLY INSECTICIDE DECAY RATE BY IRS DISTRICTS

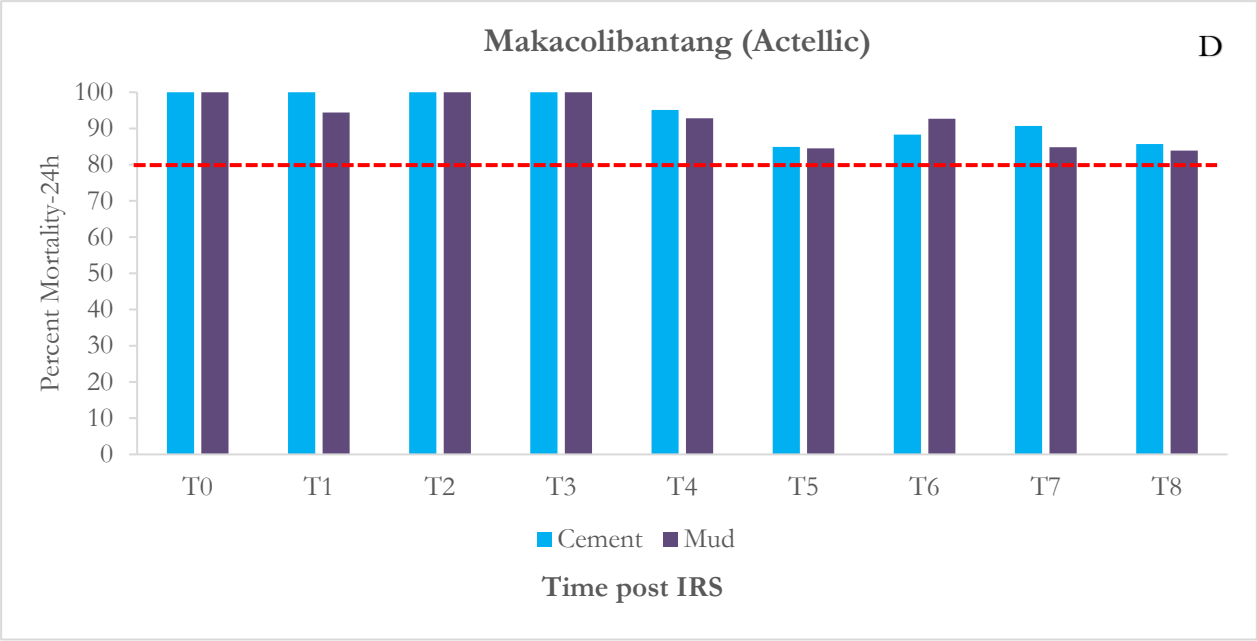
The residual efficacy of insecticide was evaluated each month for eight months in the four IRS districts (July to February 2023) (Figure 33). The results indicate that all insecticides showed a high residual efficacy with an average mortality rate above 99%, on all wall types tested (mud and cement) in Fludora Fusion and SumiShield sites and greater than 80% mortality in walls sprayed with Actellic at T8, February 2023 (Figure 33).

Figure 33: Monthly Cone Bioassay Mortality Rates of *An. coluzzii* on Fludora Fusion (Figures A, B), SumiShield (Figure C) and Actellic (Figure D) Sprayed on Mud and Cement by IRS District*









*Red dotted line represents the 80% efficacy threshold. Each bar represents % mortality up to five days for Fludora Fusion and SumiShield and to 24h for Actellic over successive months.

3.6 *An. stephensi* in Urban Area of Dakar and Touba

3.6.1 Diversity and distributions of larval habitats

Anopheles larval habitats were surveyed in September and November 2022. The team sampled 35 habitats, of which 14 were positive for *Anopheles* larvae (Table 17). These larval habitats identified were mostly natural larval

habitats in Dakar (6/10) and Touba (4/4) (Figure 37). *Anopheles* larval habitats were found most frequently in puddles in Dakar (6/10) and ponds in Touba (2/4).

Table 17: Positivity of *Anopheles* Larval Habitats, by Type and Study Sites, September and November 2022

Type of larval habitats	Dakar							Touba		
	Port and surroundings				Airport and surroundings					
	N	p	n		N	p	n	N	p	n
Basins	0	-	-		1	0	1	2	0	2
Ponds	0	-	-		0	-	-	6	2	4
Puddles	3	3	0		3	3	0	6	1	5
Drain channels	0	-	-		0	-	-	1	1	0
Water tank	3	1	2		3	0	3	0	-	-
Car tire	2	1	1		0	-	-	2	0	2
Abandoned car	1	1	0		0	-	-	0	-	-
Abandoned canoe	1	1	0		0	-	-	0	-	-
Concrete mixing	0	-	-		1	0	1	0	-	-
Total	**10**	**7**	**3**		**8**	**3**	**5**	**17**	**4**	13

Key: N=number of water bodies prospected; p=number of water bodies positives for *Anopheles* larvae, n=number of water bodies negatives for *Anopheles* larvae.

Figure 37: *Anopheles* Larval Habitats in Dakar (A, B, C, D) and Touba (E, F, G, H)

Of the 1,526 larvae collected in Dakar, 665 (44%) were morphologically identified as *Anopheles* (Table 18). Larvae were collected from all site which were positive with *Anopheles* The larvae collected were mostly composed of early stages of larvae (L1 & L2) at the airport while at the port, the late stages of larvae (L3 & L4) were more abundant. This is also what resulted in a difference in the emergence rates recorded at the Port (55%) and at the airport (37%) (Table 18).

Table 18: Total *Anopheles* Collected and Identified in Dakar and Touba during the *An. stephensi* Survey, September and November 2022

Collection months	Dakar (+ sites=10)						Touba (+ sites=4)		
	Port and surroundings			Airport and surroundings					
	LC	AE	ID	LC	AE	ID	LC*	AE	ID
September 2022	458	215	215	633	130	130	-	70	70
November 2022	100	90	90	335	230	230	-		
Total	**558**	**305**	**305**	**968**	**360**	**360**	**189**	**70**	**70**

Key: LC=number of larval collected, AE=number of adults emerged, ID= morphologically identified anopheles.
*The number of larvae collected by month was not recorded, only the total for Touba sites.

3.6.2 MORPHOLOGICAL AND MOLECULAR IDENTIFICATION OF *AN. STEPHENSI* LARVAL SURVEY

The morphological identification carried out on 735 *Anopheles* adults from the larval habitats shows only the presence of *An. gambiae* s.l.. Molecular identification carried out on all 735 samples revealed the presence of 718 *An. arabiensis* and 17 negatives. Seventeen (17) samples that did not amplified will be DNA quantified using a nanodrop before re-analyzed.

4. Discussion and Conclusion

4.1 Species Composition and Vector Density

Eight different *Anopheles* species (*An. gambiae* s.l., *An. funestus* s.l., *An. pharoensis*, *An. rufipes*, *An. nili*, *An. coustani, An. ziemanni,* and *An. squamosus*) were collected. *An. gambiae* s.l. was predominant within the country, followed by *An. rufipes* and *An. nili. An. squamosus* and *An. nili* were found only in the Sudano-Guinean zone. *An. funestus* s.l. was mainly collected in the Sahelian zone, which is close to a river with vegetation. Using PSC, *An. rufipes* was the second highest species collected after *An. gambiae* s.l. No *An. nili* or *An. coustani* were collected with this method, which can be explained by their high exophilic trends. With HLC, *An. pharoensis* was the second most numerous species collected in the Sudano-Sahelian and Sudanese zones. *An. nili* was collected only in *the* Sudano-Guinean zone, using HLC. CDC-LT was the only method that recorded fewer *An. gambiae* s.l. than *An. ziemanni* and *An. funestus* in the Sahelian zone, justifying the ability of this method to collect various and non-endophagic mosquitoes.

Through species identification of *An. gambiae* via PCR*, An. arabiensis* represented the main vector species collected in all geographical zones except the Sudano-Guinean zone. Moreover, *An. gambiae* was the most frequent species collected during the rainy season, especially in the Sudano-Guinean districts. *An. coluzzii* appeared only during the rainy season, except in the Sahelian zone, where it was absent. Only *An. funestus* s.s. was molecularly identified among the group collected in the Sudano-Sahelian, Sudanese, and Sudano-Guinean zones. The species composition and trends were similar to those in the previous year's reports, particularly with *An. arabiensis* as the main vector in the larger part of the country.

4.2 Vector Biting Behavior, Indoor Resting Status, and Parity Rates

The different number of HLC sites across the different geographical zones did not allow zone comparison. However, in the sites where HLCs were performed (Sudano-Sahelian, Sudanese, and Sudano-Guinean), HBRs were higher only in the Sudano-Guinean zone, with more than 20 b/p/n. In other zones, HBRs were less than 1 b/p/n. A trend of exophagic *An. gambiae* s.l. was found in the Sudanese and Sudano-Guinean zones. The higher density of *An. gambiae* s.l. was recorded in the middle of the rainy season (August and September), except in the Sudano-Sahelian zone, where the highest density was in October. Overall, *An. gambiae* s.l. HBRs were higher in the second half of the night indoors than outdoors.

At all IRS sites, *An. gambiae* s.l. bite more indoors than outdoors, except in Koumpentoum, where the indoor/outdoor trends were similar. In control sites, outdoor biting rates were higher than indoor in Tambacounda and Salémata but were similar in Malem Hodar. The indoor biting behavior in IRS sites shows the appropriateness of the strategy in the selected sites for malaria vector control. This finding will support the NMCP for IRS advocacy and continuity. However, the IRD trends observed in the IRS sites could not support any density reduction conclusion as the spraying was conducted at the beginning of the rainy season where the number of mosquitoes increases with different trends per site specific.

Despite having the highest HBR, the Sudano-Guinean zone recorded the lowest IRD of *An. gambiae* s.l. and the highest proportion of blood-fed females. The IRD decreased progressively from the Sahelian to the Sudano-

Guinean zone. This can be explained by the presence of favorable outdoor resting places with more vegetation from the north to the south of the country.

Prokopack aspirators were used to collect mosquitoes resting outdoors in areas where the exophilic trend was observed previously. Only a few *Anopheles* were collected and only three species found (*An. gambiae* s.l., *An. funestus*. And *An. rufipes*). *An. gambiae* s.l. was predominant, with more than 50% of gravid and semi-gravid females. This collection method did not allow us to collect enough *Anopheles* to describe where exophilic mosquitoes rest outside.

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, which are less likely to be infected. The almost equal proportion of parous and nulliparous vectors among the dissected mosquitoes in IRS districts may be due to the impact of IRS, which is meant to reduce older vectors. About 52% of the vectors from IRS sites were parous. In contrast, the control sites reported a slightly higher parity rate (60%) than the IRS sites did.

4.3 Malaria Transmission Indicators

The highest anthropophilic rate was recorded in the Sudano-Guinean zone, where *An. gambiae* was predominant, with a mean AR of ≥75%. In the other geographical zones, where *An. arabiensis* was predominant, AR was very low, specifically in the Sahelian zone, where it was less than 5%. *An. funestus* was collected with high density only in the Sudano-Sahelian zone, where its preference was for equine.

Only *An. gambiae* s.l. was found positive for *P. falciparum* sporozoites, and none of the *An. funestus* females were found to be positive. Infected *An. gambiae* s.l. was noted only in the Sudanese and Sudano-Guinean zones, which corresponds to regions where malaria incidences are higher in country. In the four IRS districts, it was only in Kédougou where infected females were found (1.13%), but the infection rate was significantly higher in its control (Saraya/Salémata: 2.58%). In other IRS districts (Koumpentoum and Makacolibantang), infected females were also found in the control site of Tambacounda district (Lycounda: 0.49%). Of the infected females, none was *An. arabiensis* or *An. melas*. The higher infection rate was from the *An. coluzzii*/*An. gambiae* hybrid (5%), followed by *An. gambiae* (2%) and *An. coluzzii* (1%). These results are in concordance with the species trophic behavior described below.

The level of the transmission was related to the distribution of *An. gambiae* s.l. species and IRS intervention. Transmission was higher in areas where *An. gambiae* was the main malaria vector and was not perceptible in areas where *An. arabiensis* was predominant. In IRS districts of Koungheul, Koumpentoum, and Makacolibantang, infected vectors were not detected, except in Kédougou in the Sudano Guinean zone, where it remained but less than in its controls. With only *An. gambiae* recording higher AR and infection than other members of the complex, and the main vector as *An. arabiensis* in most of the geographical zones and sites, malaria transmission is likely to be controlled if efforts could be put in to target and reduce *An. gambiae* vector populations in areas where highest densities were recorded during the rainy season.

Suitable *An. stephensi* larval habitats were found in Dakar around the seaport and airport and in Touba, through larval collections performed in September and November 2022. However, these collections only yielded *An. gambiae* s.l. that was identified both morphologically and molecularly as *An. arabiensis*. Surveys need to be maintained and spread, if possible, to monitor and report whenever and wherever the species is found.

4.4 Remanence of Spayed Insecticides

Eight months after spraying, all IRS insecticides were still present and effective on both mud and cement walls. The results are similar to most of the country and as previously reported in IRS campaigns. This confirms that all IRS insecticides last for at least eight months in Senegal, though Actellic disappeared more quickly than did clothianidin-based insecticides.

4.5 Insecticide Susceptibility

Resistance to all three tested pyrethroids was recorded, though pre-exposure to PBO reversed the resistance status in most of the sites and for different pyrethroids. PBO + deltamethrin was effective in Koungheul, Koumpentoum, Makacolibantang, and Tambacounda; PBO + permethrin in Koungheul and Koumpentoum; and PBO + alpha-cypermethrin in Koungheul and Koumpentoum. However, in sites where the status was not reversed to susceptibility, a substantial increase in mortality was always recorded after pre-exposure to PBO. Furthermore, the pyrethroid resistance observed in all *An. gambiae* s.l. in all sites was mostly low and moderate, especially with deltamethrin. High resistance to alpha-cypermethrin was reported only in Malem Hodar and to permethrin only in Tambacounda. Full susceptibility was also recorded against chlorfenapyr in all sites and geographic zones. The vector profile against all tested insecticides will guide the NMCP for ITN-related decision-making for effective vector control. However, any resistance management plan put in place in the country should consider the increase and spread of the *kdr* east in the country for vector control decision making to avoid fixation of both mutations across the country. This will require advocating for complementary measures using different insecticides other than pyrethroid-based tools.

For insecticides used in IRS, *An. gambiae* populations tested were susceptible to clothianidin and pirimiphos-methyl, which were used in 2022. For bendiocarb, populations tested were also susceptible even in areas where resistance was detected in the past. Furthermore, the *Ace*-1 mutation allele was still absent or very low in all districts, conforming to the use of organophosphate and carbamate-based insecticides for vector control strategy such as IRS.

4.6 Conclusion

Overall, the vector populations of all surveyed sites were found to have a slight trend towards outdoor biting, which is similar to previous year's trends. Outdoor biting has always been a cause for concern in the country, as currently implemented vector control strategies focus on indoor biting and resting behaviors. The majority of the vectors surveyed in country are *An. arabiensis*. The data gathered during the 2022 entomological activities will continue to support the NMCP in malaria vector control decision-making. The activities in the surveyed sites will help better characterize and understand vector behavior for appropriate vector control implementation and management to accelerate the fight against malaria and towards elimination in Senegal.

5. RECOMMENDATIONS

- Outdoor biting was observed at many sites. Consider larval source management as a complementary vector control intervention which targets both indoor and outdoor biting mosquitoes to help reduce vector population densities. A first step would be to identify areas where it would be feasible to conduct these studies to have measurable impact.
- Due to the continued resistance of local vectors to pyrethroid insecticides, consider expanding the use of piperonyl butoxide ITNs or introducing dual active ingredient ITNs, especially where ITNs are the only vector control intervention. Given the susceptibility to chlorfenapyr observed at all sites, a dual active ingredient pyrethroid-chlorfenapyr based ITN will be appropriate to deploy as recommended by the WHO[1], either nationwide or in areas of high pyrethroid resistance.
- Extend community-based surveillance as a resource-effective approach to close gaps in areas of Senegal where the entomology profile needs to be updated can support efforts towards subnational tailoring of vector control interventions.
- To help improve VectorLink Collect management and the scale-up of mobile data collection, add an entomology database manager to the project country team for permanent monitoring of the dashboard and capacity-building at UCAD for use of the system.
- Work to integrate/migrate VectorLink Collect into DHIS2 and used nationally and at the regional and district levels for decision making when possible.
- Given the challenges with collecting sufficient mosquitoes using Prokopack aspirators, focus on re-training collectors to ensure they are using the equipment effectively, to look for other likely mosquitoes resting sites and consider deploying this collection method in other sites.

3 1 The new WHO recommendation (2023) is that Pyrethroid-Chlorfenapyr ITN can be deployed instead of pyrethroid-only long last insecticide treated nets for prevention of malaria in adults and children in areas with pyrethroid resistance

ANNEX A: *ANOPHELES* SPECIES COMPOSITION AND BEHAVIOR

TableA1: *Anopheles* species composition collected across the country using HLC, CDC LT and PSC From April through December 2022

Geographical Zone	District	*An. gambiae s.l.*	*An. funestus s.l.*	*An. rufipes*	*An. pharoensis*	*An. nili*	*An. coustani*	*An. ziemanni*	*An. squamosus*	Total species
Sahelian	Keur Momar Sarr	31	121	5	0	0	0	32	0	189
	Richard Toll	117	0	0	12	0	0	5	0	134
	Sub total	148	121	5	12	0	0	37	0	323
Sudano-Sahelian	Diourbel	417	0	1	1	0	0	1	0	420
	Koungheul	249	0	20	13	0	0	0	0	282
	Malem Hodar	68	1	4	2	0	0	0	0	75
	Touba	71	**0**	0	0	0	0	0	0	71
	Sub total	805	1	25	16	0	0	1	0	848
Sudanese	Koumpentoum	66	0	9	3	0	0	0	0	78
	Makacolibantang	110	0	25	1	0	0	0	0	136
	Tambacounda	979	5	86	44	0	0	0	0	1,114
	Sub total	1,155	5	120	48	0	0	0	0	1,328
Sudano-Guinean	Kédougou	1,399	2	7	1	4	6	1	2	1,422
	Salemata	825	7	21	0	194	4	5	0	1,056
	Saraya	339	8	3	1	13	0	0	0	364
	Velingara	217	7	85	12	0	3	10	0	334
	Sub total	2,780	24	116	14	211	13	16	2	3,176
	TOTAL	**4,888**	**151**	**266**	**90**	**211**	**13**	**54**	**2**	**5,675**

Table A2: *Anopheles* Species Composition by Geographical Zone Collected Using PSC From April through December 2022

Geographical Zone	District	*An. gambiae s.l.*	*An. funestus s.l.*	*An. rufipes*	*An. pharoensis*	*An. ziemanni*	*An. squamosus*	Total
Sahelian	Keur Momar Sarr	31	89	3	0	2	0	125
	Richard Toll	116	0	0	4	0	0	120
	Sub total	147	89	3	4	2	0	245
Sudanese	Koumpentoum	52	0	8	2	0	0	62
	Makacolibantang	87	0	25	1	0	0	113
	Tambacounda	529	5	85	23	0	0	642
	Sub total	668	5	118	26	0	0	817
Sudano-Guinean	Kédougou	19	0	6	0	0	1	26
	Salemata	29	1	21	0	0	0	51
	Saraya	13	1	3	0	0	0	17
	Velingara	139	3	80	0	0	0	222
	Sub total	200	5	110	0	0	1	316
Sudano-Sahelian	Diourbel	289	0	1	0	0	0	290
	Koungheul	184	0	20	0	0	0	204
	Malem Hodar	58	1	4	0	0	0	63
	Touba	48	0	0	0	0	0	48
	Sub total	579	1	25	0	0	0	605
Total		**1,594**	**100**	**256**	**30**	**2**	**1**	**1,983**

Table A3: *Anopheles* Species Composition by Geographical Zone Collected Using CDC LT From September through December 2022

Geographical Zone	District	*An. gambiae s.l.*	*An. funestus s.l.*	*An. rufipes*	*An. pharoensis*	*An. ziemanni*	*An. coustani*	Total
Sahelian	Keur Momar Sarr	0	32	2	0	30	0	64
	Richard Toll	1	0	0	8	5	0	14
	Sub total	1	32	2	8	35	0	78
Sudano-Sahelian	Diourbel	128	0	0	1	1	0	130
	Touba	23	0	0	0	0	0	23
	Sub total	151	0	0	1	1	0	153
Sudano-Guinean	Velingara	78	4	5	12	10	3	112
Total		230	36	7	21	46	3	343

Table A4: *Anopheles* species composition collected across the country using HLC From April through December 2022

		Indoor									Outdoor									Total Indoor + Outdoor								
Zone	**District**	***AG s.l.***	***AF s.l.***	***AN***	***AP***	***AC***	***AZ***	***AR***	***AS***	***Total***	***AG s.l.***	***AF s.l.***	***AN***	***AP***	***AC***	***AZ***	***AR***	***AS***	***Total***	***AG s.l.***	***AF s.l.***	***AN***	***AP***	***AC***	***AZ***	***AR***	***AS***	***Total***
Sudano-Sahelian	Kounghoul	31	0	0	9	0	0	0	0	40	34	0	0	4	0	0	0	0	38	65	0	0	13	0	0	0	0	78
	Malem Hodar	7	0	0	0	0	0	0	0	7	3	0	0	2	0	0	0	0	5	10	0	0	2	0	0	0	0	12
	Subtotal	**38**	**0**	**0**	**9**	**0**	**0**	**0**	**0**	**47**	**37**	**0**	**0**	**6**	**0**	**0**	**0**	**0**	**43**	75	0	0	15	0	0	0	0	90
Sudanese	Koumpentoum	7	0	0	0	0	0	0	0	7	7	0	0	1	0	0	1	0	9	14	0	0	1	0	0	1	0	16
	Maka	10	0	0	0	0	0	0	0	10	13	0	0	0	0	0	0	0	13	23	0	0	0	0	0	0	0	23

		Indoor									Outdoor									Total Indoor + Outdoor								
Zone	**District**	***AG* s.l.**	***AF s.l.***	***AN***	***AP***	***AC***	***AZ***	***AR***	***AS***	***Total***	***AG* s.l.**	***AF s.l.***	***AN***	***AP***	***AC***	***AZ***	***AR***	***AS***	***Total***	***AG* s.l.**	***AF s.l.***	***AN***	***AP***	***AC***	***AZ***	***AR***	***AS***	***Total***
Sudano-Guinean	Colibantang																											
	Tambacounda	190	0	0	5	0	0	1	0	196	260	0	0	16	0	0	0	0	276	450	0	0	21	0	0	1	0	472
	Subtotal	207	0	0	5	0	0	1	0	213	280	0	0	17	0	0	1	0	298	487	0	0	22	0	0	2	0	511
	Kédougou	666	0	1	0	1	1	1	0	670	714	2	3	1	5	0	0	1	726	1380	2	4	1	6	1	1	1	1396
	Salemata	415	3	87	0	2	4	0	0	511	381	3	107	0	2	1	0	0	494	796	6	194	0	4	5	0	0	1005
	Saraya	169	1	4	1	0	0	0	0	175	157	6	9	0	0	0	0	0	172	326	7	13	1	0	0	0	0	347
	Subtotal	1250	4	92	1	3	5	1	0	1356	1252	11	119	1	7	1	0	1	1392	2502	15	211	2	10	6	1	1	2748
Total		**1495**	**4**	**92**	**15**	**3**	**5**	**2**	**0**	**1616**	**1569**	**11**	**119**	**24**	**7**	**1**	**1**	**1**	**1733**	**3064**	**15**	**211**	**39**	**10**	**6**	**3**	**1**	**3349**

AG: ***An. gambiae,*** AF***: An. funestus*** AN***: An. nili*** AP***: An. pharoensis*** AC ***: An. coustani*** AZ ***: An. ziemani*** AR ***: An. rufipes*** AS ***: An. squamosus***

Table A5: *Anopheles* species composition collected IRS districts and controls using HLC and PSC From April through December 2022

Geographical Zone	District	*An. gambiae s.l.*	*An. funestus s.l.*	*An. rufipes*	*An. pharoensis*	*An. nili*	*An. coustani*	*An. ziemanni*	*An. squamosus*	Total
Fludora Fusion	Koungheul	249	0	20	13	0	0	0	0	282
Control	Malem Hodar	68	1	4	2	0	0	0	0	75
	Subtotal	**317**	**1**	**24**	**15**	**0**	**0**	**0**	**0**	357
Fludora Fusion	Koumpentoum	66	0	9	3	0	0	0	0	78
Actellic	Makacolibantang	110	0	25	1	0	0	0	0	136
Control	Tambacounda	979	5	86	44	0	0	0	0	1,114
	Subtotal	**1,155**	**5**	**120**	**48**	**0**	**0**	**0**	**0**	1,328
Sumishield	Kédougou	1,399	2	7	1	4	6	1	2	1,422
Control	Salemata	825	7	21	0	194	4	5	0	1,056
	Saraya	339	8	3	1	13	0	0	0	364
	Subtotal	**2,563**	**17**	**31**	**2**	**211**	**10**	**6**	**2**	**2,842**
Total		**4,035**	**23**	**175**	**65**	**211**	**10**	**6**	**2**	**4,527**
Total IRS districts		**1,824**	**2**	**61**	**18**	**4**	**6**	**1**	**2**	**1,918**
Total control districts		**2,211**	**21**	**114**	**47**	**207**	**4**	**5**	**0**	**2,609**
Total IRS and controls		**4,035**	**23**	**175**	**65**	**211**	**10**	**6**	**2**	**4,527**

Table A6: *An. gambiae* s.l. Biting Rate by Geographical Zone

Zone		*An. gambiae* s.l.	H/N	HBR		Endophagic rate
Sudano - Sahelienne	Indoor	162	180	0.9	1.0	0.44
	Outdoor	203	180	1.1		
Soudanienne	Indoor	394	456	0.9	0.9	0.48
	Outdoor	421	456	0.9		
Soudano - Guineenne	Indoor	2061	192	10.7	10.7	0.50
	Outdoor	2062	192	10.7		
Total	Indoor	2617	828	3.2	3.2	**0.49**
	Outdoor	2686	828	3.2		

Table A7: *An. gambiae* s.l. Biting Rate in IRS districts and their controls

District		*An. gambiae* s.l.	H/N	HBR	
Koungheul	Indoor	156	96	1.6	1.8
	Outdoor	196	96	2	
Koumpentoum	Indoor	35	96	0.4	0.4
	Outdoor	47	96	0.5	
Makacolibantang	Indoor	24	84	0.3	0.4
	Outdoor	36	84	0.4	
Kédougou	Indoor	851	96	8.9	9.8
	Outdoor	1033	96	10.8	
Malem Hodar	Indoor	6	84	0.1	0.1
	Outdoor	7	84	0.1	
Tambacounda	Indoor	335	276	1.2	1.2
	Outdoor	338	276	1.2	
Salemata	Indoor	692	48	14.4	13.5
	Outdoor	604	48	12.6	
Saraya	Indoor	518	48	10.8	9.8
	Outdoor	425	48	8.9	
	Total IRS + control	**5303**	**1656**	**3.2**	
IRS FF (Kédougou)	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	
	Total SS	**493**	**552**	**0.9**	
Control SS (Malem Hodar and Tambacounda)	Indoor	341	360	0.9	1
	Outdoor	346	360	1	
	Total Control	**686**	**720**	**1**	

Table A8: *An. gambiae* s.l. Indoor resting density by Geographical Zone

Geographical Zone	District	*An. gambiae* s.l.	Rooms	IRD
Sahelian	District Keur Momar Sarr	31	70	0.44
	District Richard Toll	116	70	1.66
	Subtotal	**147**	**140**	**1.05**
Sudanese	District Koumpentoum	52	160	0.33
	District Maka Colibantang	87	160	0.54
	District Tambacounda	529	480	1.10
	Subtotal	**668**	**800**	**0.84**
Sudano-Guinean	District Kédougou	19	160	0.12
	District Salemata	29	80	0.36
	District Saraya	13	80	0.16
	District Velingara	139	159	0.87
	Subtotal	**200**	**479**	**0.42**
Sudano-Sahelian	District Diourbel	289	70	4.13
	District Koungheul	184	160	1.15
	District Malem Hodar	58	160	0.36
	District Touba	48	80	0.60
	Subtotal	**579**	**470**	**1.23**
Total		**1594**	**1889**	**0.84**

Table A9: *An. gambiae* s.l. Indoor resting density in IRS districts and their controls

Zone	District	*An. gambiae* s.l.	Rooms	IRD
Sudanese	District Koumpentoum	52	160	0.33
	District Makacolibantang	87	160	0.54
	District Tambacounda	529	480	1.10
	Subtotal	**668**	**800**	**0.84**
Sudano-Guinean	District Kédougou	19	160	0.12
	District Salemata	29	80	0.36
	District Saraya	13	80	0.16
	Subtotal	**61**	**320**	**0.19**
Sudano-Sahelian	District Koungheul	184	160	1.15
	District Malem Hodar	58	160	0.36
	Subtotal	**242**	**320**	**0.76**
	Total	**971**	**1440**	**0.67**
	IRS districts	342	640	**0.53**
	Controls districts	629	800	**0.79**
	Fludora districts	236	200	**1.18**
	Fludora controls	587	640	**0.92**
	SumiShield districts	19	160	**0.12**
	SumiShield controls	42	160	**0.26**
	Actellic District	87	160	**0.54**
	Actellic District	529	480	**1.10**
	IRS and controls	971	1440	**0.67**

ANNEX B: LABORATORY

Table B1: *An. gambiae* s.l. and *An. funestus* composition in the geographical zones (A to December 2022)

Geographical zones	*An. arabiensis* (%)	*An. coluzzii* (%)	*An. gambiae* (%)	*An. melas* (%)	*Hybrid An. gambiae/An. coluzzii* (%)
Sahelian	69 (100)		0		0
Sudano-Sahelian	414 (85,18)	67 (13,79)	3 (0,62)	2 (0,41)	0
Sudanese	381 (64,69)	73 (12,39)	131 (22,24)		4 (0,68)
Sudano-Guinean	171 (11,15)	125 (8,15)	1215 (79,2)		23 (1,5)

Table B2: *An. gambiae* s.l. species composition in the surveyed districts (January to December 2022)

Districts	*An. gambiae s.l.* (%)						*An. funestus ss* (%)
	# *Unamplified*	*An. arabiensis*	*An. coluzzii*	*An. gambiae*	*Hybrid An. gambiae/An. coluzzii*	*An. melas*	
Richard toll	3	69 (100.0)	0	0	0	0	0
Keur Momar_Sarr	2	15 (100.0)	0	0	0	0	43 (100.0)
Touba	1	50 (100.0)	0	0	0	0	0
Diourbel	15	245 (100.0)	0	0	0	0	0
Koungheul	5	67 (51.94)	59 (45.74)	2 (1.55)	0	1 (0'77)	1 (100.0)
Malem Hodar	3	37 (80.44)	7 (15.22)	1 (2.17)	0	1 (2.17)	0
Tambacounda	19	330 (64.33)	52 (10.14)	127 (24.75)	4 (0.78)	0	2 (100.0)
Koumpentoum	5	31 (72.09)	11 (25.58)	1 (2.33)	0	0	0
Makacolibantang	2	20 (60.61)	10 (30.3)	3 (9.09)	0	0	0
Kédougou	11	50 (7.31)	43 (6.29)	579 (84.65)	12 (1,75)	0	1 (100.0)
Salemata	17	60 (12.07)	18 (3.62)	415 (83.5)	4 (0,81)	0	3 (100.0)
Saraya	21	41 (16.21)	13 (5.14)	193 (76.28)	6 (2.37)	0	1 (100.0)
Velingara	13	20 (20.62)	51 (52.58)	25 (25.77)	1 (1.03)	0	0
Total	117	1041	264	1346	27	2	51

Annex B3: Monthly Frequencies of *An. gambiae* s.l. species collected by HLC and PSC from the geographical zones surveyed (January to December 2022)

Geographical zones	Species	January (%)	February (%)	March (%)	April (%)	June (%)	July (%)	August (%)	September (%)	October (%)	November (%)
Sahelian											
	An. arabiensis				0	0	0	11 (100)	24 (100)	33 (100)	1 (100)
Sudano-Sahelian											
	An. arabiensis				48 (100)	11 (24,45)	29 (61,7)	23 (100)	54 (94,74)	97 (85,09)	152 (100)
	An. coluzzii					33 (73,33)	17 (36,17)	0	3 (5,26)	14 (12,28)	0
	An. gambiae					0	0	0	0	3 (2,63)	0
	An. melas					1 (2,22)	1 (2,13)	0	0	0	0
Sudanese											
	An. arabiensis				21 (95,5)	18 (90)	18 (54,55)	4 (10,53)	127 (50,2)	84 (73,68)	109 (100)
	An. coluzzii				1 (4,55)	2 (10)	6 (18,18)	13 (34,21)	39 (15,42)	12 (10,53)	0
	An. gambiae				0	0	7 (21,21)	21 (55,26)	87 (34,39)	16 (14,04)	0
	Hybrid An. gambiae/An. coluzzii				0	0	2 (6,06)	0	0	2 (1,75)	0
Sudano-Guinean											
	An. arabiensis				10 (100)	14 (42,43)	4 (0,86)	16 (5)	20 (5,39)	78 (27,56)	29 (58)
	An. coluzzii					10 (30,3)	19 (4,07)	20 (6,25)	22 (5,93)	42 (14,84)	12 (24)
	An. gambiae					9 (27,27)	434 (92,93)	280 (87,5)	325 (87,6)	159 (56,18)	8 (16)
	Hybrid An. gambiae/An. coluzzii					0	10 (2,14)	4 (1,25)	4 (1,08)	4 (1,41)	1 (2)

Table B4: Blood meal sources of *An. gambiae* s.l. collected by PSCs and Prokopack mosquito aspirator per districts from April to November 2022

Species	Districts	T	ND	ID	Mono-specific					MiX			AR
					H	B	S	C	Ho	MIX H/A	MIX H/A/A	MIX A/A	
An. funestus													
	Keur Momar Sarr	34	13	21	1	5	5	0	10	0	0	0	0.048
	Tambacounda	3	1	2	0	1	1	0	0	0	0	0	0
	Salemata	1	0	1	0	1	0	0	0	0	0	0	0
	Saraya	1	0	1	0	0	1	0	0	0	0	0	0
***An. gambiae* s.l.**													
	Richard Toll	63	29	36	0	8	1	0	23	1	0	1	0.028
	Keur Momar Sarr	11	4	8	2	0	0	0	4	0	0	1	0.250
	Touba	21	1	21	9	1	2	0	7	1	0	0	0.476
	Diourbel	134	57	84	25	1	22	2	20	2	0	5	0.321
	Koungheul	90	27	67	13	2	4	0	40	1	0	3	0.209
	Malem Hodar	31	9	24	3	0	0	0	17	1	0	1	0.167
	Koumpentoum	24	4	24	0	0	4	0	13	0	1	2	0.042
	Makacolibantang	9	0	9	6	0	3	0	0	0	0	0	0.667
	Koussanar	10	4	7	1	2	1	0	1	0	0	1	0.143
	Lycounda)	36	6	37	4	0	0	1	18	6	0	1	0.270
	Tambacounda PBO-LLINs	139	16	130	87	1	6	3	19	2	0	5	0.685
	Kédougou	6	0	6	5	1	0	0	0	0	0	0	0.833
	Salemata	18	5	13	6	6	1	0	0	0	0	0	0.462
	Saraya	11	0	11	11	0	0	0	0	0	0	0	1
	Velingara	62	12	50	43	2	2	1	2	0	0	0	0.860

* Community-based mosquito surveillance/ Pyrethrum spray catch, T= tested; Id=Number of hosts successfully identified; ND= Not determined (blood meals were negative for all the antibodies tested).
H= Human; B= Bovine; S= Sheep (Ovine); C= Chicken, Ho= Horse (Equine); H/A= Human/Animal; A/A= Animal/Animal; AR= Anthropophilic rate

Table B5: Monthly Infection Rate of *An. gambiae* s.l. (January to December 2022)

District	January		February		March		April		June		July		August		September		October		November	
	T	P	T	P	T	P	T	P	T	P	T	P	T	P	T	P	T	P	T	P
Richard toll	-	-	-	-	-	-	-	-	1	0	-	-	12	0	4	0	-	-	1	0
Keur Momar Sarr	-	-	-	-	-	-	-	-	4	0	-	-	11	0	1	0	-	-	-	-
Diourbel	-	-	-	-	-	-	20	0	-	-	11	0	41	0	20	0	1	0	93	0
Touba	-	-	-	-	-	-	1	0	7	0	2	0	4	0	4	0	9	0	11	0
Koungheul	-	-	-	-	-	-	6	0	10	0	8	0	-	-	7	0	13	0	3	0
Malem Hodar	-	-	-	-	-	-	3	0	1	0	1	0	2	0	3	0	1	0	-	-
Koumpentoum	-	-	-	-	-	-	1	0	-	-	1	0	-	-	12	0	1	0	-	-
Makacolibantang	-	-	-	-	-	-	-	-	-	-	5	0	2	0	3	0	3	0	1	0
Tambacounda	-	-	-	-	-	-	7	0	2	0	7	0	42	1	176	0	86	1	82	0
Kédougou	-	-	-	-	-	-	3	0	5	0	203	2	198	1	198	1	92	4	6	0
Salemata	-	-	-	-	-	-	-	-	11	0	186	6	106	4	98	2	108	4	15	1
Saraya	-	-	-	-	-	-	-	-	1	0	71	1	57	3	104	0	53	0	6	0
Velingara	-	-	-	-	-	-	3	0	6	0	8	0	16	0	27	0	35	0	9	0

Table B6: Infection Rate of *An. pharoensis* and *An. nili* by Geographic Zone (January to December 2022)

Geographical zones	Districts	*An. pharoensis*			*An. nili*		
		T	P	CSI	T	P	CSI
Sahelian	Richard Toll	24	0	0			
	Diourbel	1	0	0			
	Koungheul	12	0	0			
	Malem Hodar	2	0	0			
Sudanese	Koumpentoum	1	0	0			
	Makacolibantang						
	Tambacounda	17	0	0			
	Tambacounda (PBO-LLINs-site) *	3	0	0			
Sudano-Guinean	Kédougou	2	0	0	2	0	0
	Salemata				138	**1**	0
	Saraya	1	0	0	12	0	0
	Vélingara	11	0	0			

Table B7: *Plasmodium falciparum* infection rate of the *An. gambiae* s.l. species collected by HLC in the IRS-District and their control (January to December 2022)

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*	5											
Keur Momar Sarr	1											
Touba	24											
Diourbel	107	0										
Tambacounda (PBO-LLINs-site) *	60			57	1	**0,02**	15			1		
Velingara*	9			4			23			1		

Table B8: Indoor and Outdoor Entomological Inoculation Rate of *An. gambiae* s.l. Females in the Surveyed Sites (January to December 2022)

Geographic zones	District	Indoor			Outdoor			Total		
		HBR	CSI	EIR	HBR	CSI	EIR	HBR	CSI	EIR
Sahelian	Richard Toll*									
	Koungheul	0,262			0,33			0,298		
	Malem Hodar	0,095			0,04			0,065		
Sudanese	Koumpentoum	0,083			0,1			0,089		
	Makacolibantang	0,036			0,14			0,089		
	Tambacounda (Controls)	1,19			1,68	0,01	**0,017**	1,435	0,0044	**0,006**
	Tambacounda PBO	0,512			0,62	0,0116	**0,007**	0,565	0,0065	**0,004**
Sudano-Guinean	Kédougou	7,917	0,0114613	**0,091**	8,52	0,0112	**0,095**	8,22	0,0113	**0,093**
	Velingara*									
	Salemata	9,143	0,0295203	**0,270**	9,81	0,0356	**0,349**	9,476	0,0324	**0,307**
	Saraya	4	0,0065359	**0,026**	3,71	0,0217	**0,081**	3,857	0,0137	**0,053**

HBR=Human biting rate, CSI=Circumsporozoite index, EIR=Entomological inoculation rate

ANNEX C: SUSCEPTIBILITY TEST DATA

Table C1: Insecticide Susceptibility Testing Activities by District 2022

Geographical zone	Districts	Chlorfenapyr	Clothianidin	Deltamethrin			Permethrin			Alpha-cypermethrin			PBO + Delta	PBO + Perm	PBO + Alpha	Bendiocarb	Pirimiphos - methyl
		100 µg/ml	4 µg/ml	1X	5X	10X	1X	5X	10X	1X	5X	10X					
SAHELO-SUDANESE	Keur Massar	100	100	21				66		7			92		96		99
	Dakar Ouest				80	100	8		94					64		98	
SUDANO-SAHELIAN	Touba	100	100	17						27			91		94	99	
	Malem Hodar	100	100	49	72	98	10	61	91	52	42	76	97	44	77	100	100
	Koungheul	100	100	91	91.3	100	82	90		17	80	100	100	95	99	99	100
SUDANESE	Koumpentoum	100	100	85	95.5	96.2	28	78	96	68	61		100	78	98	100	
	Makacolibantang	100	100	92		100		94	100	6	70	100	100		96	100	
	Tambacounda	100	92.7	40	97.1	100	11	43	76	15	22	95	98	100	90	99	100
SUDANO GUINEAN	Kédougou	100	100	88	93	97.2	38	66	100	47	51	98	93	95	91	100	100
	Vélingara	100	100	83			63			55			94	88	83	100	100

Susceptible Probable Resistant

Tests not performed

ANNEX D: BIBLIOGRAPHY

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