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THE PMI VECTORLINK PROJECT

MALI ANNUAL ENTOMOLOGICAL MONITORING REPORT JANUARY 2021 –DECEMBER 2021

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MALI ANNUAL ENTOMOLOGICAL MONITORING REPORT JANUARY 2021 –DECEMBER 2021

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ACRONYMS

b/p/n	Bite Per Person Per Night
b/p/m	Bite Per Person Per Month
b/p/cp	Bite Per Person Per Collection Period
CDC	Center For Diseases Control & Prevention
CBS	Community-Based Surveillance
CS	Capsule Suspension
CSP	Circumsporozoite Protein
EIR	Entomological Inoculation Rate
ELISA	Enzyme-Linked Immunosorbent Assay
f/r	Female Per Room
HBR	Human Biting Rate
ib/p/cp	Infected Bite Per Person Per Collection Period
HLC	Human Landing Catch
IG2	Interceptor Second Generation
IRS	Indoor Residual Spraying
IRD	Indoor Resting Density
ITN	Insecticide-Treated Net
LBMA	*Laboratoire de Biologie Moléculaire Appliquée*, Applied Molecular Biology Laboratory
LT	Light Trap
NMCP	National Malaria Control Program
PBO	Piperonyl Butoxide
PCR	Polymerase Chain Reaction
PMI	President's Malaria Initiative
PSC	Pyrethrum Spray Catch
SB	Soluble Bag/Sachet
WG	Wettable Granules
WHO	World Health Organization
WP	Wettable Powder

EXECUTIVE SUMMARY

The US President' malaria Initiative (PMI) VectorLink Mali project conducted the 2021 indoor residual spraying (IRS) campaign from June 7th, 2021, to July 16th, 2021, in the districts of Mopti and Djenné and, from June 28th to August 10th, 2021, in Bandiagra District. Actellic® 300CS was sprayed in Mopti District, SumiShield® 50WG in Djenné District and Fludora® Fusion WP-SB in Bandiagara District.

Spray quality assurance was conducted within one week of the start of the campaign and subsequent monthly insecticide residual efficacy was conducted using WHO cone bioassays on sprayed walls in each of the three IRS districts (Mopti, Bandiagara and Djenné). In Bandiagara district where Fludora Fusion was sprayed, cone bioassays were conducted using both a susceptible lab colony and from wild mosquitoes emerged from larval collections. A community-based surveillance (CBS) pilot was launched in August 2020 in two sites in Mopti district (one sprayed, one unsprayed) using CDC light traps (CDC LT) set indoors and continued until April 2021. From May 2021, CBS using CDC LT and pyrethrum spray catch (PSC) was extended to six sites in the three IRS districts. For each District, a sprayed site, and a control site (unsprayed) were allocated, and both were monitored simultaneously.

Two districts in the Sikasso region were monitored using human landing catch (HLC) and PSC for the second consecutive year. Selingué received Interceptor® G2 insecticide-treated nets (IG2 ITNs) in June 2020 and Bougouni received pyrethroid-only ITNs. Insecticide susceptibility tests were also conducted in all eight sites to inform the choice of insecticide for future IRS and ITN campaigns.

Cone bioassays performed within one week after treatment demonstrated a good spray quality with 100% mortality recorded on all types of walls sprayed with all insecticides. Actellic 300CS sprayed in Mopti showed a residual efficacy of at least six months on all the types of walls. In Bandiagara the residual efficacy of Fludora Fusion WP-SB was eight months with the susceptible *An. coluzzii* strain (Ngousso) and at least four months with wild pyrethroid-resistant *An. gambiae* s.l. The cone assays using wild collected mosquitoes could not be conducted after four months due to the dry season. In Djenne, sprayed with SumiShield 50WG, the residual efficacy was still above 80% after eight months for all the types of walls monitored. The results showed that all three IRS insecticides lasted enough time to cover the entire malaria transmission season.

Four *Anopheles* species (*An. gambiae* s.l, *An. funestus* s.l., *An. pharoensis* and *An. rufipes*) were collected in the IRS sites. *Anopheles gambiae* s.l. was the predominant *Anopheles* species collected using HLC, PSC and CDC LT in all sites (94-100% at each site). *Anopheles funestus* s.l. was found in the IRS sites (Mopti region) but not in the Sikasso region (ITN sites) during the 2021 surveillance. The peak indoor resting densities occurred generally in September in all six sites in Mopti region (sprayed and unsprayed). However, the peak indoor resting density (IRD) was recorded in July in the sprayed site of Bandiagara which was related to the postponement of IRS until after the peak density period due to the delayed delivery of the Fludora Fusion as a result of COVID-19 related supply chain disruptions. In Mopti district, an unexpected higher IRD was recorded in the sprayed site compared to the unsprayed site throughout the collection period. This could be a weakness of CBS strategy requiring close supervision for good quality of the data collected. Furthermore, the engagement of the community collectors and their availability represent other issues during the implementation of CBS.

In the Sikasso sites, where no IRS was implemented, the peak IRD was observed in September in Bougouni (IG2 nets) and October in Selingue (standard nets). In Bougouni and Selingue where HLC was conducted, the peak hourly human biting rate (HBR) was observed between 02:00 am and 03:00 am in indoor and 03:00am-04:00am outdoor in Bougouni while both indoor and outdoor peaks were recorded between 02:00am and 03:00am in Selingue. The highest entomological inoculation rate (EIR) was recorded in

Bougouni with 0.29 or infective bites per person per night (ib/p/n) 44.40 infective bites per person per the collection period (ib/p/cp) covering June to November 2021), followed by Seligue with 0.19 ib/p/n which is equal to 29.4 ib/p/cp. The EIR was zero ib/p/n in both sites of Bandiagara. In Djenné (unsprayed) and Madiana sprayed districts, the EIR was overall relatively low with 0.02 ib/p/n (3.8 ib/p/cp) and 0.06 ib/p/n (13.6 ib/p/cp) respectively over the collection period from May to December 2021. In Mopti Region, the highest EIR was observed in the sprayed site of Souforoulaye with 0.05 ib/p/n (11.6 ib/p/cp compared to the unsprayed site of Sarema (0.01 ib/p/n representing 1.7 ib/p/cp).

Anopheles gambiae s.l. was resistant to the three pyrethroid insecticides (permethrin 0.75%, deltamethrin 0.05%, and alpha-cypermethrin 0.05%) tested in all sites. Pre-exposure to PBO substantially increased mortality against all pyrethroids (mean of 22.6% to 64.1% for alpha-cypermethrin, 22.9% to 66.3% for deltamethrin and 13.7% to 34.4% for permethrin) showing the involvement, of oxidase enzymes in the insecticide resistance of the vectors. High pyrethroid resistance intensity was recorded at all sites, except in Mopti and Selingue where deltamethrin resistance was moderate.

Susceptibility to clothianidin was recorded in the three IRS districts, supporting the continued use of clothianidin-based insecticides for IRS. *An. gambiae* s.l was tested to both chlorfenapyr doses 100 µg/bottle and 200 µg/bottle in the seven sites monitored. Susceptibility was recorded in three (Koulikoro, Mopti and Selingue) of the seven sites at the dose of 100 µg/bottle while the remaining sites recorded susceptibility at 200 µg/bottle, except Djenné. Chlorfenapyr tests will need to be repeated in these districts during the next year's susceptibility data collections to confirm the trends observed.

The data collected can be used to support decisions around any future IRS campaign in the strategic deployment for different insecticides and guide NMCP in on introducing new generation ITNs for upcoming mass ITN distribution campaigns.

1. INTRODUCTION

Malaria vector control in Mali primarily depends on nationwide use of insecticide-treated nets (ITNs) and indoor residual spraying (IRS) in targeted high transmission areas. The US President's Malaria Initiative (PMI) supported annual IRS campaigns in Mali since 2008. Initially implemented in Koulikoro Region, IRS operations were relocated to Mopti in 2017 based on its high malaria prevalence rate. Between 2017 and 2020, four consecutive IRS campaigns were conducted in different districts of the country (Bandiagara, Djenné and Mopti) while the prevalence decreased from 60% to 25% during the same period in Mopti Region compared to 19% national average (EDS 2018).

In 2020, the NMCP conducted a nationwide ITN mass distribution campaign in which Interceptor G2 (IG2) ITNs were distributed in four districts in Sikasso Region.

The primary objective of the VectorLink Mali project in 2021 was to continue to reduce malaria-associated morbidity and mortality in three districts of Mopti Region (Mopti, Bandiagara, and Djenné) through IRS. As recommended by the Mali NMCP vector control policy, an insecticide rotation strategy was established for resistance management. In 2021 Actellic® 300CS was used in Mopti, SumiShield® 50WG in Djenné and Fludora® Fusion WP-SB in Bandiagara. In 2021 IRS campaign, Fludora Fusion WP-SB was used in Mali for the second time and for the first time in Bandiagra. Over the period of June 7th to August 10th, 2021, the project sprayed a total of 61,791 structures out of 63,907 eligible structures found by SOPs, resulting in a spray coverage rate of 96.7% despite the highly unstable security conditions and ongoing COVID-19 pandemic in many of the targeted areas.

Furthermore, VectorLink Mali conducted entomological monitoring activities to evaluate the impact of vector control interventions put in place in sentinel sites selected by the NMCP. Entomological surveillance is a key component of integrated vector control programming, providing information on malaria vector density, behavior, and transmission parameters in sites where vector control interventions are implemented. Activities included vector surveillance in eight sites across two regions and insecticide resistance monitoring in seven sites. The data collected will continuously support the NMCP and the malaria vector control stakeholders in determining the optimal timing and insecticides for IRS and inform the selection of ITNs for mass distribution campaigns.

2. METHODOLOGY

2.1 STUDY AREA

The community-based surveillance (CBS) pilot study that started in 2020 in two sites (Toguel and Sarema) in Mopti District ended in April 2021. In 2020, Sarema was sprayed with Fludora Fusion and Toguel was not sprayed. Collection in those two sites were done using only CDC LT. Data collected from August to December 2020 were presented in the 2020 VectorLink Mali Annual Entomological Report (The PMI VectorLink Project Mali, Annual Entomological Monitoring Report. January - December 2020). The present report includes results of the pilot from January to April 2021 for Toguel and Sarema.

Starting in May 2021, longitudinal vector surveillance was conducted in six sites located in the three IRS districts (two sites per district). Sarema was not sprayed in 2021 and was therefore used as a control site. In the Sikasso region, a longitudinal vector surveillance using HLC, and PSC was conducted in a district covered by IG2 nets (Selingue) and a neighboring district covered by a standard pyrethroid-only Yorkool ITNs (Bougouni) (Table 1).

Insecticide resistance monitoring was also conducted in eight sites including three current IRS districts (Mopti, Bandiagara, and Djenné), one former IRS district (Koulikoro), two sites in Sikasso Region where IG2 net and Yorkool net were distributed in 2020 (Selingue and Bougouni) and two other sites (Kita and Niono) chosen in collaboration with the NMCP (Figure 1).

Figure 1: Map of Mali Showing Regional Boundaries and 2021 Entomological Surveillance Sites

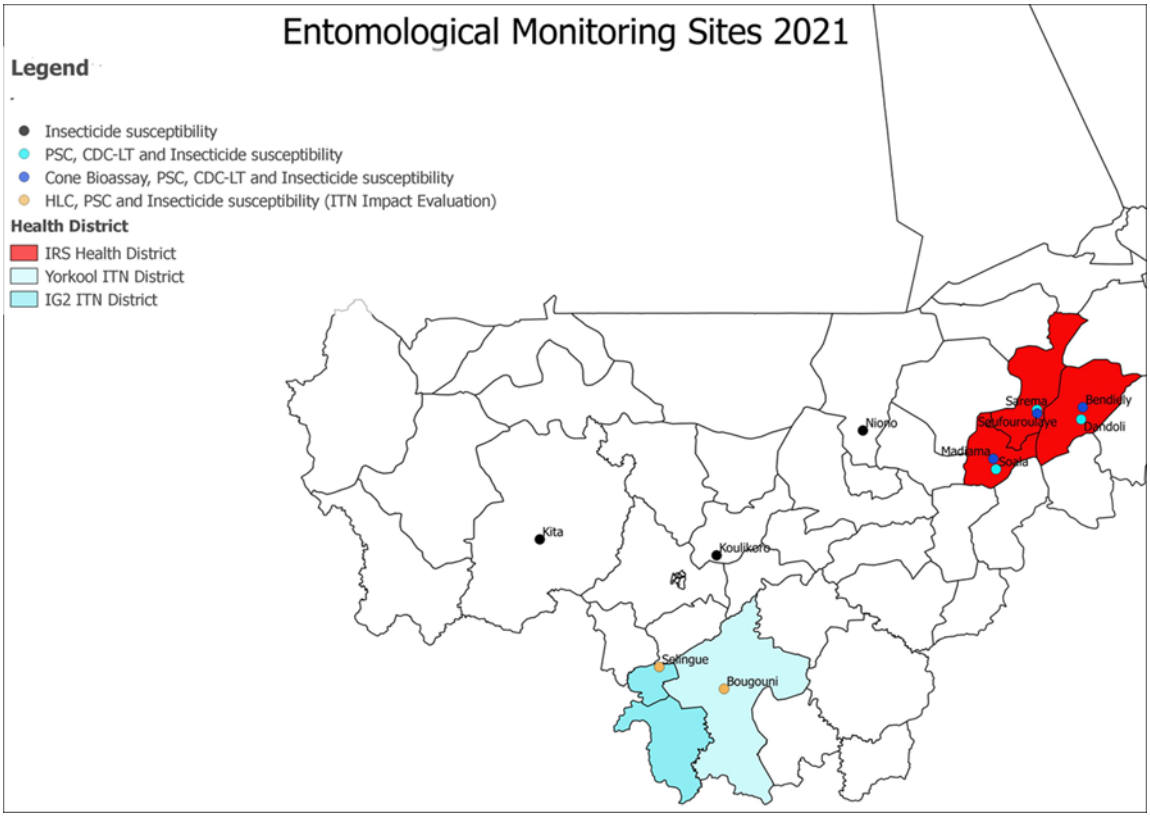


Table 1: Monthly Entomological Surveillance Sites

Region	District	Health Area	Site (village)	Spray Status	Trapping Period	Trapping Methods	IRS History or ITN Mass Campaign
Mopti	Mopti	Socoura	Sarema	Sprayed in 2020	January to April 2021	CDC LT	CBS Pilot sites
		Toguel	Toguel	Unsprayed			
Mopti	Mopti	Souforoulaye	Soufouroulaye	Sprayed	May to December 2021	CDC LT & PSC	AC 2017, SS 2018, AC 2019, FF 2020. PYR ITN 2020 & AC 2021.
		Socoura	Sarema	Unsprayed			
	Bandiagara	Bendieli	Bendieli	Sprayed			AC 2017, 2018, 2019, SS 2020, PYR ITN 2020 & FF 2021
		Bandiagara central	Dandoly	Unsprayed			
	Djenné	Madiama	Madiama	Sprayed			AC 2017, SS 2018, SS 2019, AC 2020, PYR ITN 2020 & SS 2021.
		Soala	Soala	Unsprayed			
Sikasso	Selingue	Tinko	Gueleba 2	Unsprayed	July to November 2021	HLC & PSC	Interceptor G2 Net
	Bougouni	Bougouni sud	Dalabani	Unsprayed			Yorkool Net

Note: AC = Actellic 300CS (organophosphate), SS = SumiShield 50WG (Neonicotinoid), FF = Fludora Fusion WP-SB (Neonicotinoid + deltamethrin), PYR = pyrethroid.

2.1.1 Longitudinal Monitoring

All entomological monitoring was conducted according to standard VectorLink standard operating procedures (SOPs) (Tools and Innovations – Vector Link (pmivectorlink.org)).

2.1.2 Community-based Surveillance (CBS)

In 2021, the CBS approach was extended to six sites (one sprayed and one unsprayed site in each of the three IRS districts in Mopti region) from May to December 2021. Due to the extremely high numbers of mosquitoes collected in 2020, the collections were adjusted to three houses per night for eight nights per month (24 different houses per month vs 2020 where 60 houses per month were sampled) using CDC LT and three houses per day for four days per month (12 different houses per month) using PSC.

2.1.3 CDC Light Traps

The traps were installed inside of three houses, near the bed where a human bait slept, for 24 nights per month. Traps were set at about 1.5 meters from the ground and operated from 6:00pm to 6:00am. The traps were recovered the following morning and all the collected mosquitoes were stored in petri dishes with silica gel (stored in the fridge at the local health facility) and information (date, site code, number of the house, name of the chief of the household etc.) noted on the box by the community collectors. During the monthly supervision activities, entomological technicians performed morphological identification and individually preserved *Anopheles* mosquitoes in Eppendorf tubes with silica gel. Preserved mosquito samples were sent to the Laboratoire de Biologie Moléculaire Appliquée (LBMA) for biomolecular analyses.

CDC LTs are considered a proxy measure for human biting rate. Therefore, results are presented as the mean human biting rate per month according to the following calculation:

$$Mean\ human\ biting\ rate = \frac{total\ number\ of\ An.\ gambiae\ s.l.\ collected\ by\ CDC\ LT\ per\ month}{total\ number\ of\ traps\ set\ per\ month}$$

2.1.4 Human Landing Catch

In the two Sikasso ITN sites, human landing catches (HLCs) were carried out in each site from 6:00pm to 8:00am in four randomly selected houses per night for two consecutive nights each month (eight houses total per month, same houses every month) to determine the human biting rates of malaria vector species. HLC results are presented as the mean human biting rate per person per night, for each month according to the following calculation:

$$Mean\ human\ biting\ rate = \frac{total\ number\ of\ An.\ gambiae\ s.l.\ collected\ per\ month}{total\ number\ of\ trap\ nights\ per\ month}$$

2.1.5 Pyrethrum Spray Catch

A commercial aerosol containing permethrin 0.25%, tetramethrin 0.20%, D-phenothrin 0.01% and PBO 0.34% was used to collect indoor resting mosquitoes using pyrethrum spray catch (PSC) in 20 houses per site per month in the ITN sites (Selingue and Bougouni). Ten houses per day were surveyed from 7:00a.m. to 10:00a.m. during two consecutive days (20 houses total per month), with the same houses used every month. In the CBS sites, PSCs were conducted in six houses per day for two days for a total of 12 houses per month. All female *Anopheles* were assessed for their abdominal status (unfed, fed, half gravid and gravid) and morphologically identified using the key of Coetzee, 2020.

PSC results are presented as the mean indoor resting density per room per day according to the following calculation:

$$Mean\ An.\ gambiae\ s.l.\ indoor\ resting\ density = \frac{total\ number\ of\ An.\ gambiae\ s.l.\ collected\ by\ PSC\ per\ day}{total\ number\ of\ rooms\ surveyed\ per\ day}$$

2.2 IRS Quality Assessment and Residual Efficacy Monitoring

The World Health Organization (WHO) wall cone and fumigant bioassays were conducted monthly, starting in the first week of IRS, to assess the quality of spray and residual efficacy of the sprayed insecticides, in the three IRS sites (Djenné, sprayed with SumiShield 50WG; Mopti, sprayed with Actellic 300CS; and Bandiagara, sprayed with Fludora Fusion WP-SB). Cone bioassays were conducted in ten structures per site for a total of 30 structures (with three cones tested per room at heights of 0.5, 1.0, and 1.5m). The same houses were used for both quality assurance and residual efficacy (SOP 09/01). An insectary-reared susceptible strain of *An. coluzzii* Ngousso was used for cone bioassays. For each cone, 10-12 female mosquitoes aged 2-5 days were exposed for 30 minutes, removed to labelled disposable cups and fed with 10% sugar solution cotton bolls for delayed mortality recording. Mortality rates were recorded 24 hours after exposure for Actellic 300CS and every 24 hours for up to five days for SumiShield 50WG and Fludora Fusion WP-SB. Negative controls consisted of at least 30 female *An. coluzzii* Ngousso exposed to an unsprayed wall in each site. Because Fludora® Fusion WP-SB contains a pyrethroid insecticide, a resistant wild *An. gambiae* s.l. strain was tested simultaneously with the susceptible Ngousso strain on walls sprayed with Fludora® Fusion WP-SB (when enough were available).

2.3 Insecticide Susceptibility Testing

2.3.1 WHO Susceptibility Tests

From July to November 2021, the VectorLink Mali team conducted insecticide resistance monitoring in eight sites (Selingue, Bougouni, Kita, Koulikoro, Niono, Mopti, Djenné and Bandiagara) across the country. Larvae and pupae of *An. gambiae* s.l. were collected in each site from several larval habitats, pooled, and reared to adulthood in the field laboratory. Insecticide susceptibility tests were conducted on 2–5-day-old adult females using WHO tube tests (SOP 06/01) and CDC bottle assays (SOP 04/01).

For each tube test, about 80–100 female *An. gambiae* s.l. were tested against the insecticide (in four batches of 20–25) and an additional 40–50 mosquitoes were tested in two control tubes (20–25 each) in parallel.

The diagnostic concentrations of permethrin (0.75%), deltamethrin (0.05%), alpha-cypermethrin (0.05%), and pirimiphos-methyl (0.25%) were tested in all sites, except for clothianidin which was only tested in the three IRS sites (Mopti, Djenné and Bandiagara). Clothianidin 2% papers were prepared locally using SumiShield and distilled water and following the protocol designed by PMI VectorLink (https://pmivectorlink.org/resources/tools-and-innovations). The susceptibility testing was conducted using WHO test kits, and the mortality was recorded up to seven days post-exposure. All the clothianidin tests were performed in the VectorLink facility in Bamako with the susceptible *An. coluzzii* strain being tested in parallel as a positive control to ensure papers were correctly impregnated.

When pyrethroid resistance was confirmed, resistance intensity was also tested at 5 and 10 times the diagnostic concentration of permethrin, deltamethrin and alpha-cypermethrin. The resistance intensity was conducted in the three IRS districts (Djenné, Selingue and Bougouni) and in the ITN districts (Selingue and Bougouni) in 2021.

Synergist assays with piperonyl butoxide (PBO) were conducted for deltamethrin, permethrin, and alpha-cypermethrin according to the WHO tube test protocol to determine the involvement of cytochrome P450s metabolic enzymes in pyrethroid resistance. A high percent mortality and/or reversal of susceptibility when pre-exposed to PBO indicates probable involvement of enzymes such as P450s in the resistance mechanism. CDC bottle assays were used for testing chlorfenapyr at the doses of 100µg/bottle and 200µg/bottle with one-hour exposure, and mortality recorded every 24 hours up to three days (72 hours). For all tests, resistance and intensity were defined following the WHO criteria (WHO 2016):

Resistance status at diagnostic doses:

- 98% or greater mortality indicates susceptibility
- Between 90 and 97% mortality indicates possible resistance
- Less than 90% mortality indicates confirmed resistance

Resistance intensity with mortality at 5x and or mortality at 10x:

- 98–100% at 5x: Low resistance
- <98% at 5x and 98–100% at 10x: moderate resistance
- <98% at 10x: high resistance

2.4 Molecular Analysis of Mosquito Samples

The abdomen, legs and wings of adult mosquitoes caught in the field were analyzed by polymerase chain reaction (PCR) for species identification according to the protocol described by Santolamazza *et al.*, 2008. This method allows identification of *An. gambiae*, *An. coluzzii* and *An. arabiensis*. The head and thorax of the same specimens collected through routine monthly CDC LT and HLC were used for circumsporozoite protein (CSP) enzyme-linked immunosorbent assay (ELISA) according to the protocol of Beier *et al.* (2002). The sporozoite rate was calculated as follows:

$$Sporozoite\ rate\ =\ \frac{total\ number\ of\ infected\ An.\ gambiae\ s.l.}{total\ number\ of\ An.\ gambiae\ s.l.\ tested\ by\ ELISA}\ x100$$

Dead and surviving mosquitoes from susceptibility tests were preserved individually in 1.5ml Eppendorf tubes containing 70% ethanol for species identification and characterization of insecticide resistance markers including, *kdr* 1014F, *kdr* 1014S and *ace1*R. The protocol of Huynh *et al*., (2007) was used to identify the presence of voltage gated sodium channel *kdr* 1014F, *kdr* 1014S mutations within the populations of *An. gambiae* s.l. The detection of the *ace-1*R mutation was done following the protocol described by Weill *et al.* (2004). The resistance allele frequency of each resistance mechanism was expressed using the formula.

$$Allele\ frequency\ =\ \frac{2n\ RR + n\ RS}{2\ (nRR + nRS + nSS)}$$

2.5 DATA MANAGEMENT

The VectorLink Mali project adopted the DHIS2-based VectorLink Collect database for entomological data management in 2020. All paper forms are entered monthly into the online platform. The entomology database manager monitors data entry and carries out quality controls for three datasets: longitudinal monitoring, insecticide resistance, and residual efficacy. The lead entomologist provides regular technical oversight throughout the data workflow. In preparation for the annual report, the Home Office Senior Entomology Advisor and Monitoring and Evaluation Specialist support a comprehensive quality review of all 2021 data before exporting. All the data presented in this report are from the VectorLink Collect database.

3. RESULTS

3.1 MALARIA VECTOR SPECIES COMPOSITION

3.1.1 ANOPHELES COLLECTED AT THE CBS PILOT SITES (MOPTI)

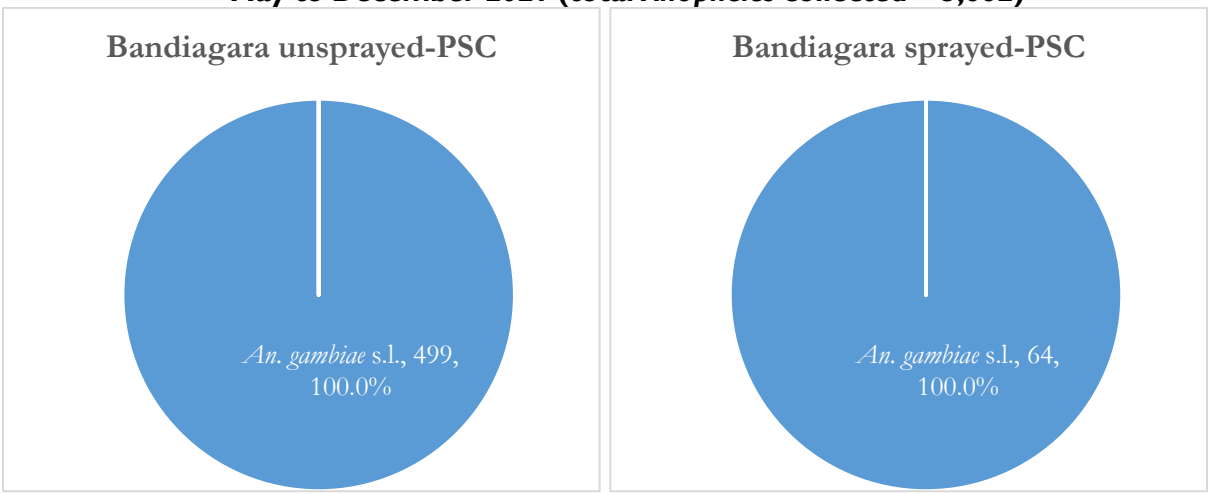
The pilot study began in August 2020 and performed until April 2021 in two sites in Mopti district (Sarema and Toguel). Between January and April of 2021, which corresponds to Mali's dry season, 256 *Anopheles* mosquitoes were collected including 248 (97.0%) *An. gambiae* s.l and 8 *An. pharoensis*. Eighty-three percent (83%, n=208) of *An. gambiae* s.l were collected in the sprayed site of Sarema and 17% (40) in the unsprayed site of Toguel.

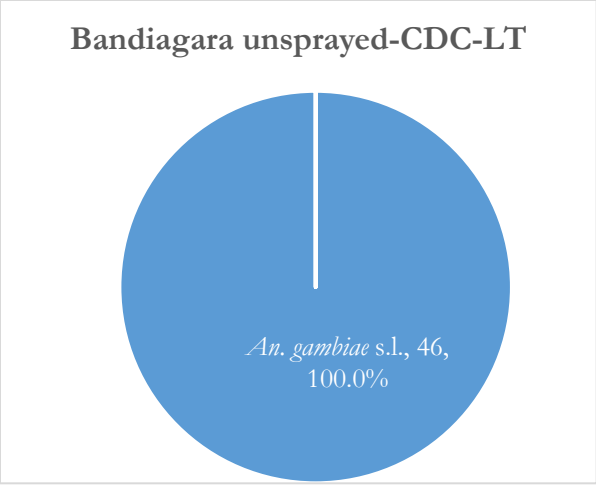
3.1.2 ANOPHELES COLLECTED AT THE IRS AND CONTROL SITES (MOPTI)

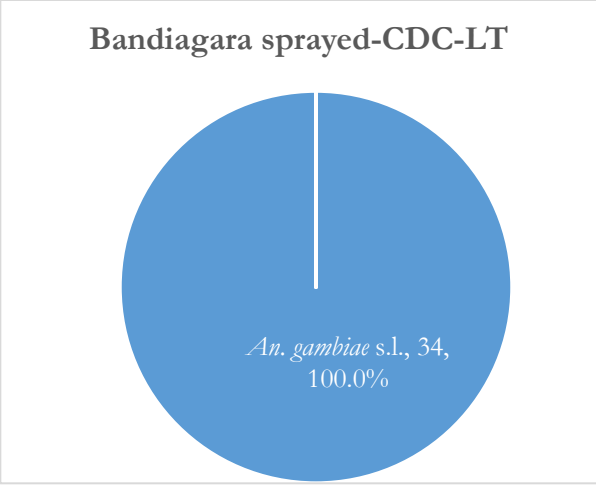
Collections from May through December 2021 yielded a total of 5,662 *Anopheles* mosquitoes using CDC LT and PSC in the six sites (three sprayed and three unsprayed) of three IRS Districts (Mopti, Djenné and Bandiagara). *Anopheles gambiae* s.l., *An. funestus* s.l., *An. pharoensis* and *An. rufipes* were collected during the monitoring period. Approximately 42.4% (2,403) of *Anopheles* were collected by CDC LT and 57.6% (n=3,259) were collected through PSC. *Anopheles gambiae* s.l was the predominant species collected, accounting for 98.8% (n=5,596) of *Anopheles* collected by CDC LT and PSC followed by *An. pharoensis*, 0.5% (n=29)) *An. rufipes 0.4% (n=26)* and *An. funestus* s.l.0.1% (11) (Figure 2).

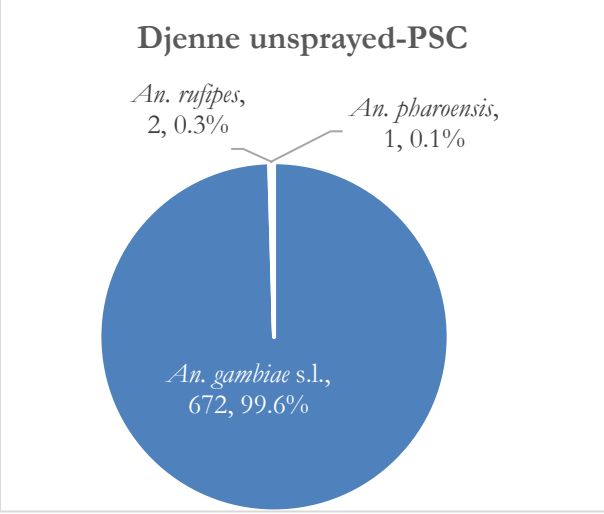
The number of *Anopheles* collected by CDC LT was generally higher in the unsprayed site of Djenné than sprayed area (unsprayed n=974 versus sprayed n=403), contrasted in Mopti with higher number in the sprayed site (825) than unsprayed (121). Only Bandiagara recorded similar density of *Anopheles* mosquitoes in sprayed (34) versus unsprayed (46) sites. Using PSC, a total of 2,062 *Anopheles* mosquitoes were collected in the IRS sites (261 in Djenne, 1,737 in Mopti, and 64 in Bandiagara) showing lower trends than in the respective unsprayed sites in Djenne (675) and Bandiagara (499), but not in Mopti (23).

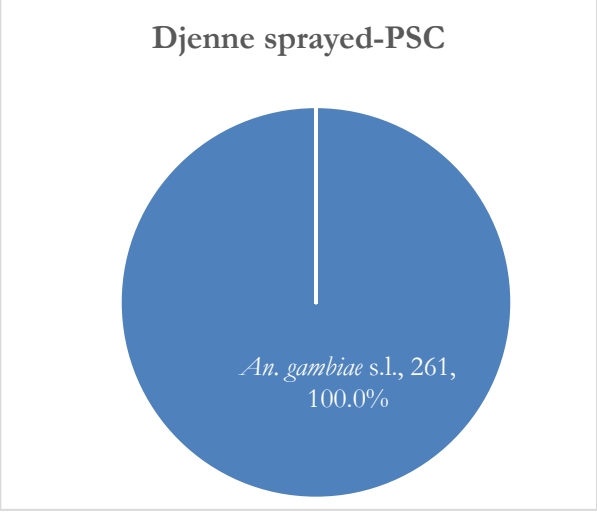
Figure 2: Species Composition of *Anopheles* Collected by CDC light trap and Pyrethrum Spray Catches in All six sites in the three IRS districts (Bandiagara, Djenné, Mopti) from May to December 2021 (total *Anopheles* collected = 5,662)

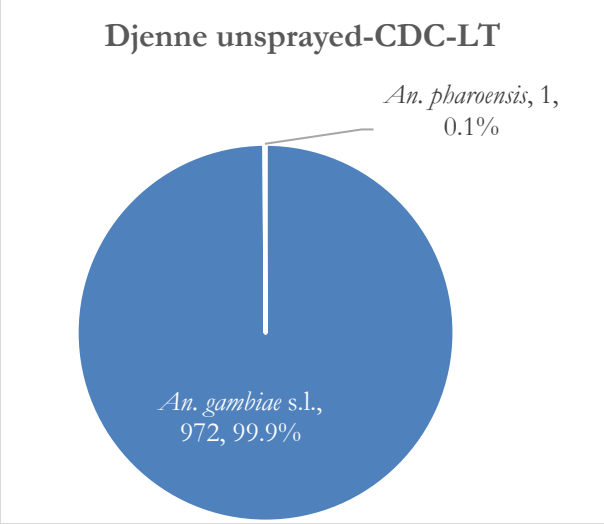


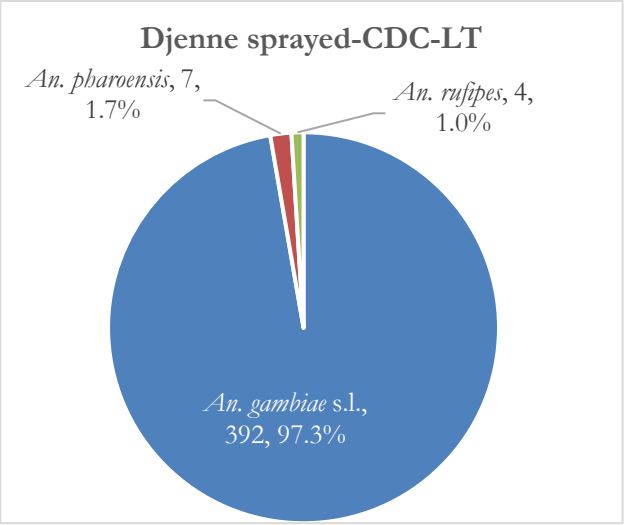

Bandiagara unsprayed-CDC-LT
An. gambiae s.l., 46, 100.0%

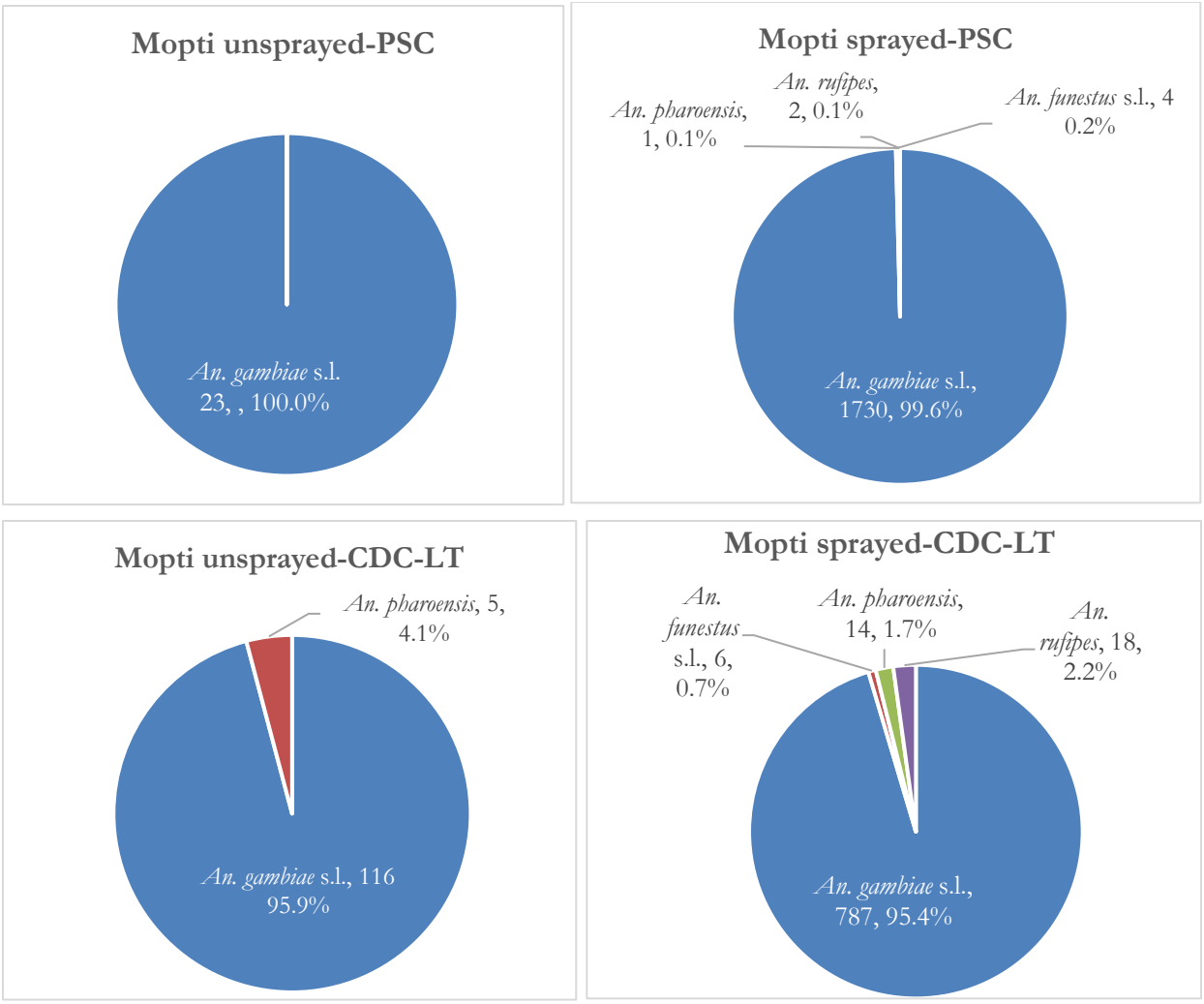

Bandiagara sprayed-CDC-LT
An. gambiae s.l., 34, 100.0%


Djenne unsprayed-PSC
An. rufipes, 2, 0.3%
An. pharoensis, 1, 0.1%
An. gambiae s.l., 672, 99.6%


Djenne sprayed-PSC
An. gambiae s.l., 261, 100.0%


Djenne unsprayed-CDC-LT
An. pharoensis, 1, 0.1%
An. gambiae s.l., 972, 99.9%

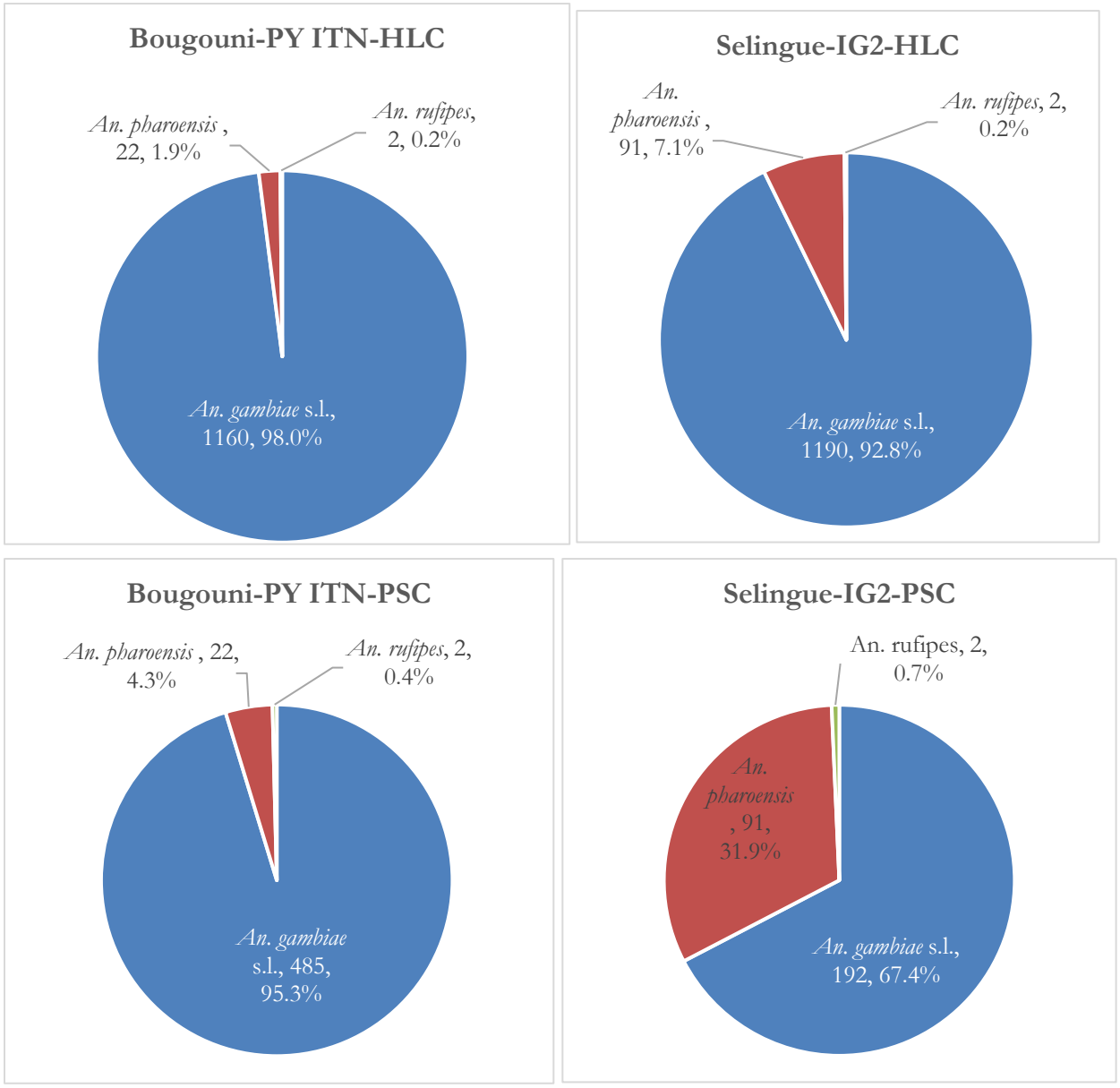

Djenne sprayed-CDC-LT
An. pharoensis, 7, 1.7%
An. rufipes, 4, 1.0%
An. gambiae s.l., 392, 97.3%



3.1.3 Anopheles collected at the ITN Distribution Sites (Sikasso)

In Selingue and Bougouni sites, a total of 3,145 *Anopheles* were collected through PSC and HLC (Figure 3). Of these, 96.2% (n=3,027) were *An. gambiae* s.l., followed by *An. pharoensis* 3,6% (n=114) and *An. rufipes* 0.12% (n=4). About 77.6% (n =2,350) of all *An. gambiae* s.l were collected by HLC and only 22.4% (677) through PSC. The number of *Anopheles* species collected was relatively similar in Bougouni (52%; n=1277) and Selingue (48%; n=1190).

Figure 3: Species Composition of *Anopheles* Collected by Human Landing Catch and Pyrethrum Spray Catch in Selingue (Interceptor G2 ITNs) and in Bougouni (pyrethroid-only ITNs) from July to December 2021 (total *Anopheles* collected = 3,145)



3.2 Indoor Resting Density

Figures 4-6 present the monthly PSC data from the six sites in the three IRS districts in the Mopti Region. The mean indoor resting density (IRD) was generally highert in the unsprayed sites compared to the sprayed sites, except in Mopti District with higher IRD in the sprayed site (Soufouroulaye) (Figure 4). In Bandiagara, the peak of mean IRD was observed in September in the unsprayed site (Dandoli) with 15.3 female *An. gambiae* s.l. per room (f/r) and in July in the sprayed site (Bendieli) with a low density 2.5 f/r (Figure 5). In the Djenne District, the peak of IRD was observed in October for Soala (unsprayed) with 27.5 f/r and in September with 9.3 f/r in Madiama (sprayed) (Figure 6). Note: All figures error bars represent the 95% confidence intervals.

Figure 4: Mean Monthly Indoor Resting Density of *An. gambiae* s.l. in MOPTI (sprayed and unsprayed) sites from May to December

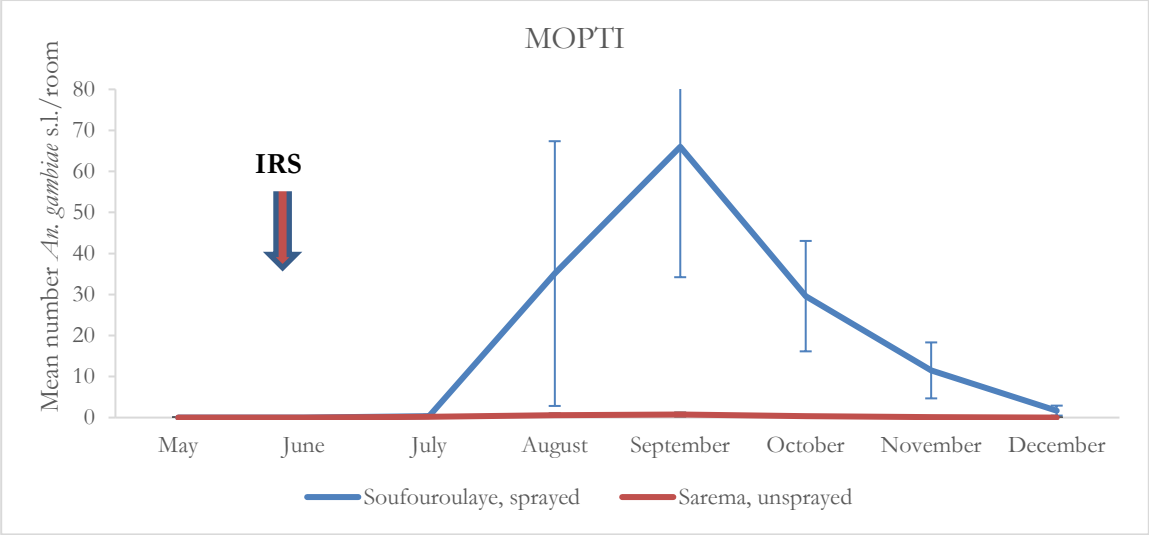


Figure 5: Mean Monthly Indoor Resting Density of *An. gambiae* s.l. in Bandiagara (sprayed and unsprayed) sites from May to December

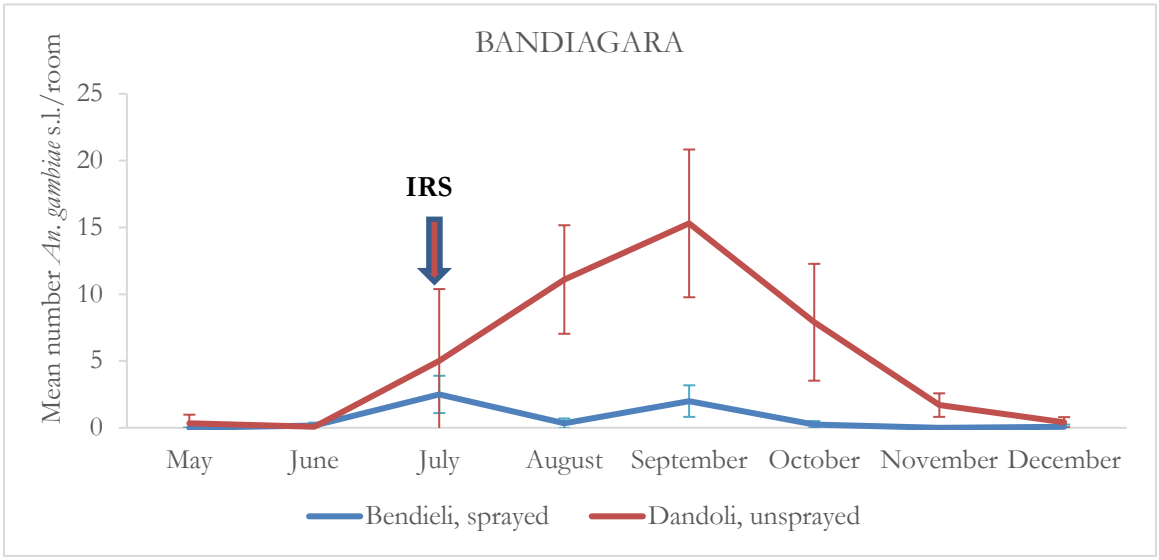
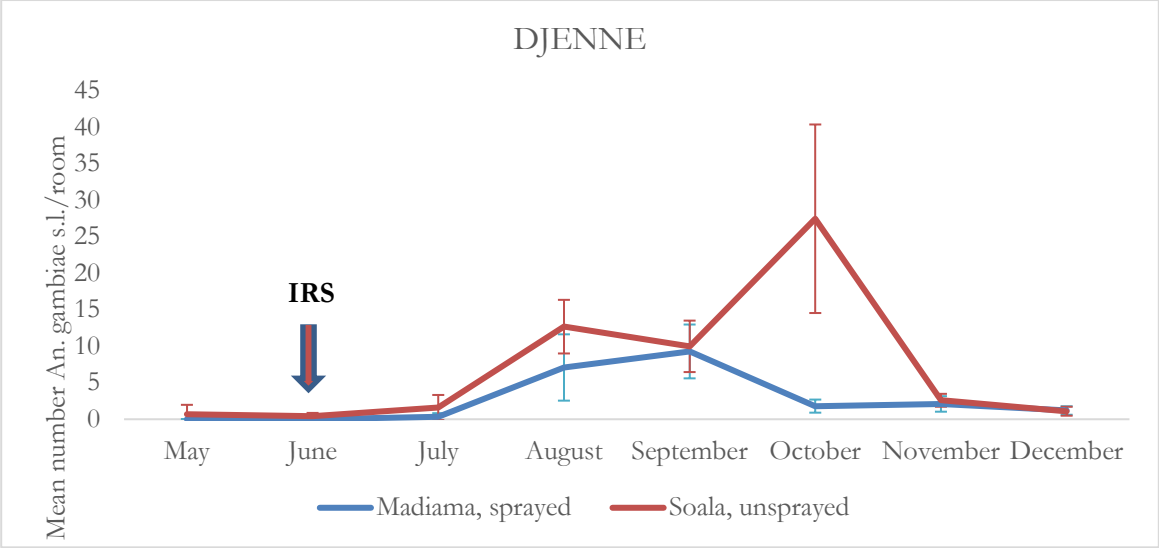
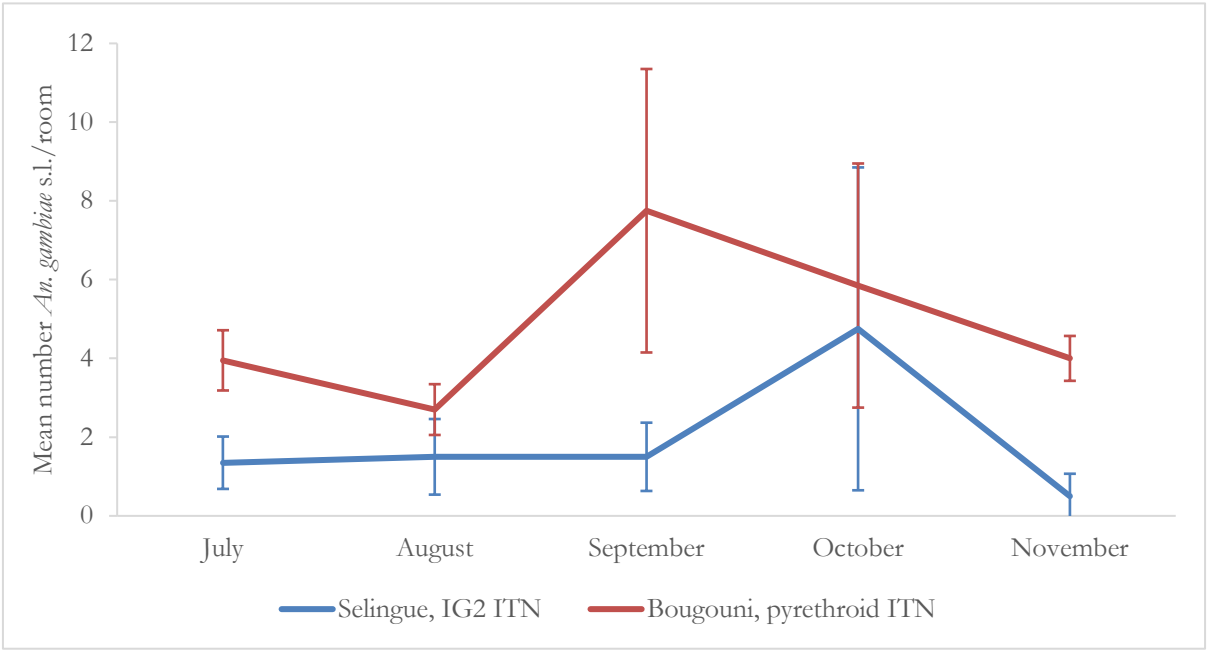


Figure 6: Mean Monthly Indoor Resting Density of *An. gambiae* s.l. in DJENNE (sprayed and unsprayed) sites from May to December



Higher peaks mean IRD was observed in Bougouni (where pyrethroid ITNs were distributed in 2020) in September with 7.75 f/r than that of Selingue (Interceptor G2) in October with 4.75 f/r (Figure 7). The mean density over the five-month monitoring period was lowest in Selingiue compared to Bougouni with respectively 1.92 f/r and 4.85 f/r.

Figure 7: Monthly *Anopheles gambiae* s.l Indoor Resting Density in Bougouni (pyrethroid ITN) and Selingue (Interceptor G2) from July to November 2021.

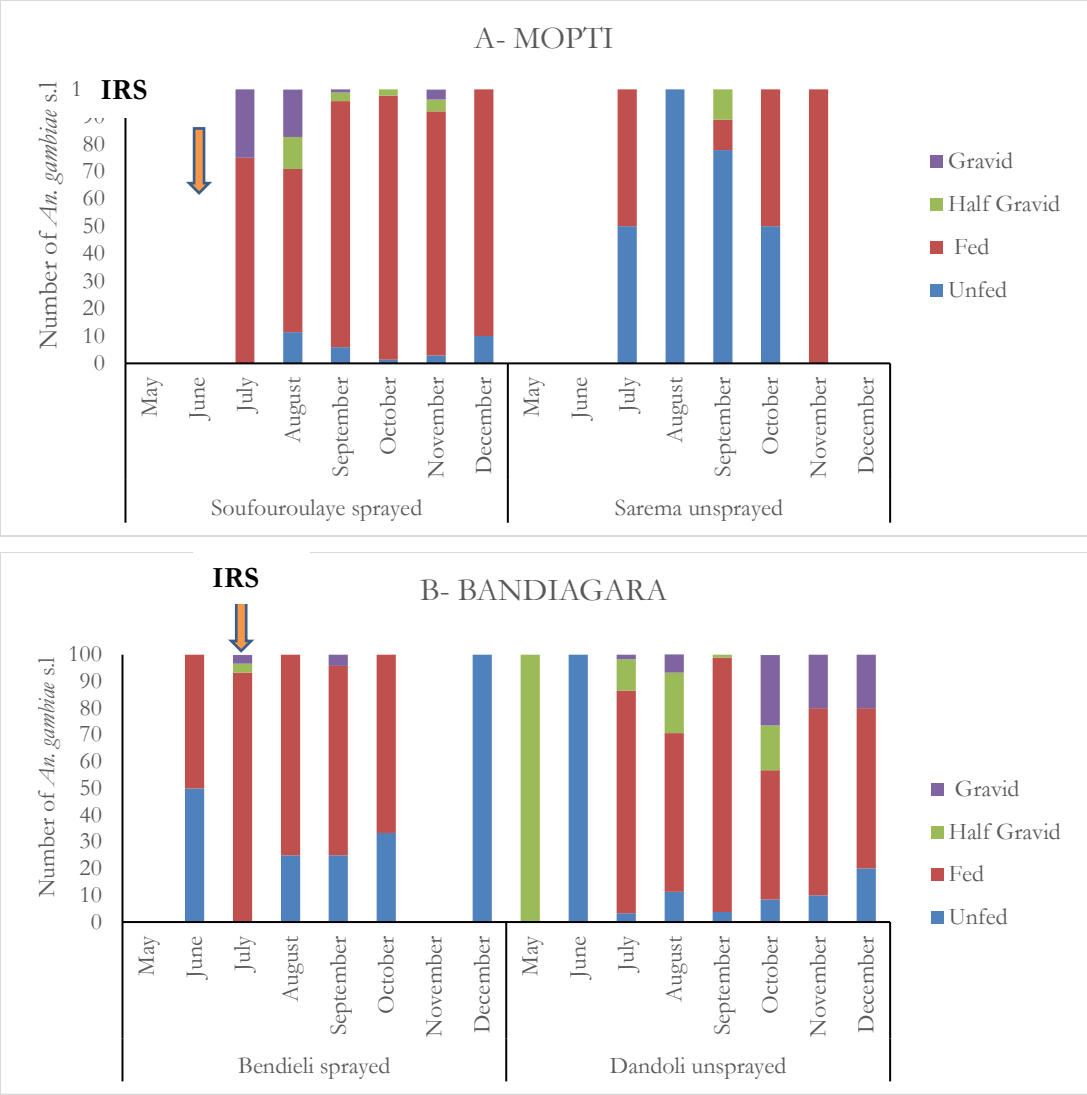


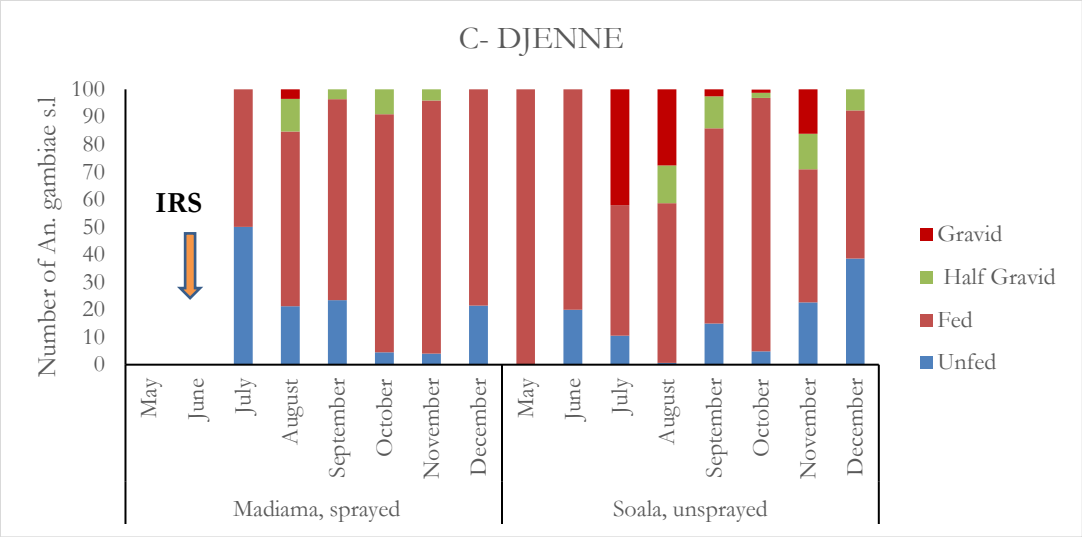
3.3 ABDOMINAL STATUS OF *AN. GAMBIAE* S.L. (COLLECTED BY PSC)

The abdominal status of *An. gambiae* s.l. collected by PSC in the six sites of the three IRS districts is presented in Figure 8. When comparing the number of fed and gravid *An. gambiae* s.l between the sprayed and the unsprayed site, most vectors collected were fed regardless of spray status in Bandiagara and Djenne. In Bandiagara, the number of half gravid and gravid females was highest in the unsprayed site from July to

December, whilst in Djenné the half gravid and gravid mosquitoes were highest in July and August in the unsprayed site. In Mopti, the fed mosquitoes were highest in the sprayed site, whilst the unfed females were highest in the unsprayed site (low number of mosquitoes collected), and the highest number of gravid mosquitoes was recorded in the sprayed site from July to September.

Figure 8: Abdominal Status of *An. gambiae* s.l. Collected by PSC from July to December 2021 in six sites in the Three IRS Districts (Mopti (A), Bandiagara (B) and Djenné (C))



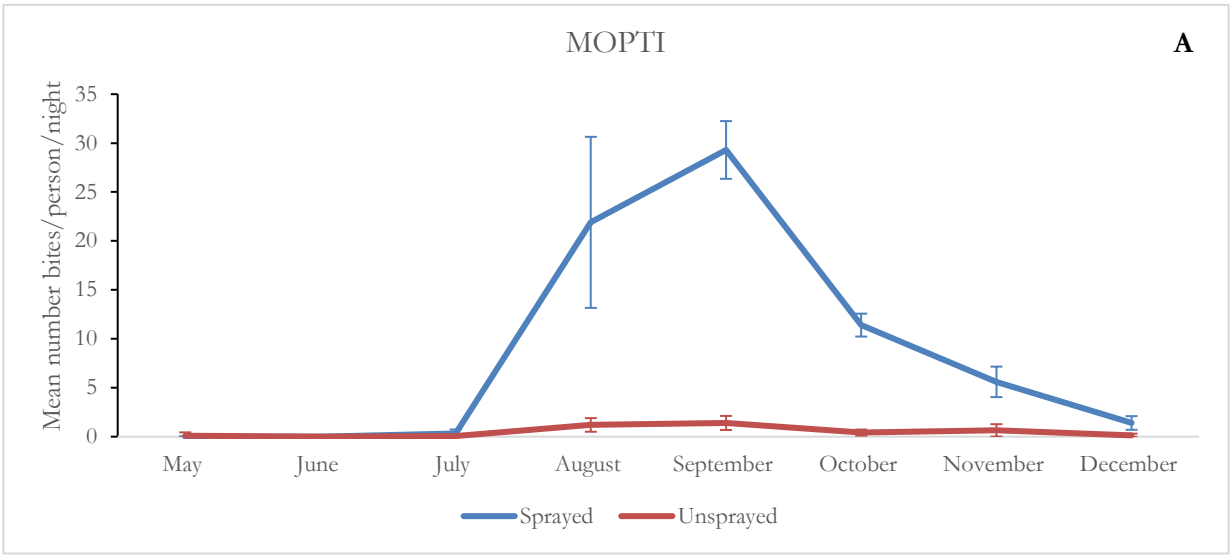


3.4 *AN. GAMBIAE* S.L. HUMAN BITING RATES

Human biting rates (HBRs) were measured as proxy with indoor CDC-LT use in CBS for the six sites in the three IRS districts (one sprayed and one unsprayed in each district) (Figure 9).

In Mopti, the peak was observed in September in the sprayed site with 29.3 b/p/n. The HBR was low (< 1.3 b/p/n) in the unsprayed site of Mopti with a very low number of *An. gambiae* s.l collected during the surveillance period (Figure 9 A). In Bandiagara, the peak HBR was observed in September in both sprayed and unsprayed site with 5.3 bites/person/night (b/p/n) observed in the unsprayed site while in the sprayed site, the HBR per month was low and remains below 1.0 b/p/n during the period (Figure 9 B). In Djenné, the peak was recorded in August in the unsprayed site with 17.0 b/p/n and in September in the sprayed site with 8.3 b/p/n (Figure 9 C).

Figure 9: Monthly Biting Rates of *An. gambiae* s.l. in the three IRS districts from May to December 2020.



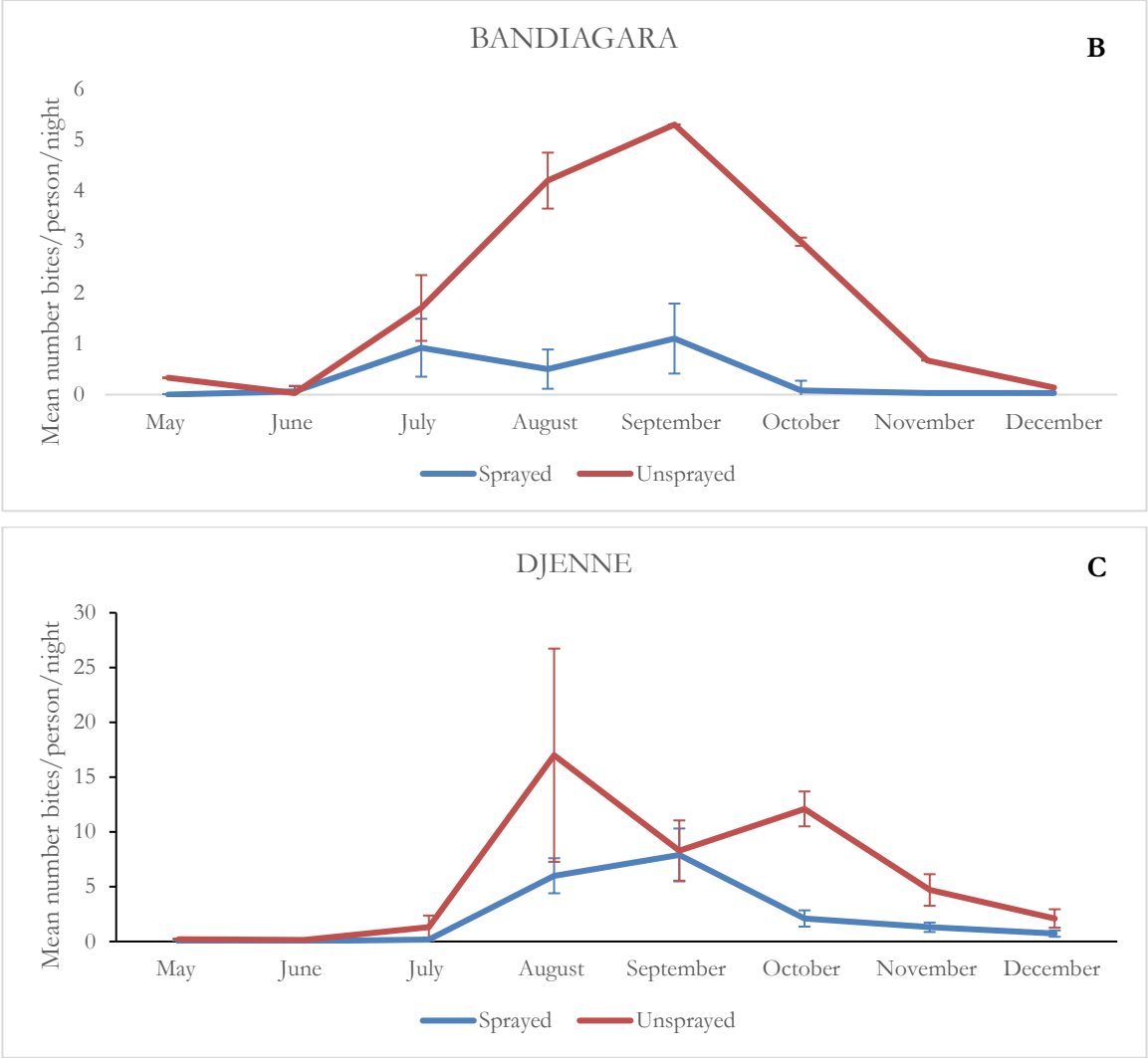
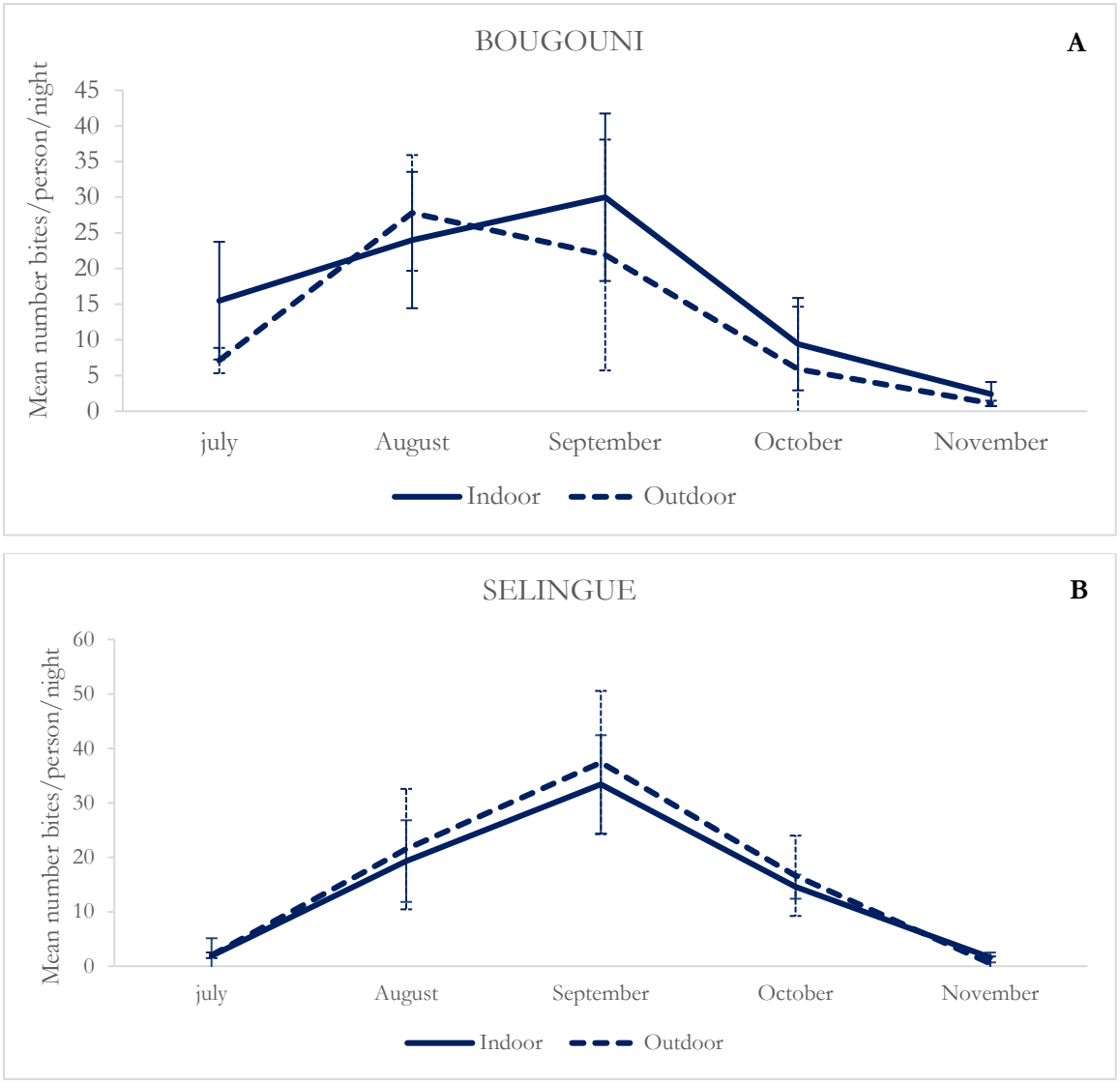


Figure 10 shows the biting rates of both sites in Sikasso Region. The peak *An. gambiae* s.l biting rate was similar in Bougouni (pyrethroid ITNs) and Selingue (Interceptor G2 ITNs). Both indoor and outdoor peaks were recorded in September in Selingue with respectively 37.4 b/p/n and 33.4 b/p/n. In Bougouni the peak indoor was observed in September with 30 b/p/n and the peak outdoor was recorded early in August with 27.8 b/p/n.

Figure 10: Monthly Indoor and Outdoor Biting Rates of *An. gambiae* s.l. in Bougouni (pyrethroid ITN) (A) and Selingue (Interceptor G2) (B) from August to December 2021



3.5 BITING TIMES OF *AN. GAMBIAE* S.L.

Figures 11 and 12 show the mean biting times of *An. gambiae* s.l. recorded using HLC indoors and outdoors in Bougouni and Selingue. The biting trends showed similar behavior with peak biting between 1:00am and 5:00am indoors and outdoors in both sites.

Figure 11: Mean *An. gambiae* s.l. Hourly Biting Rates in Bougouni (Pyrethroid ITN)

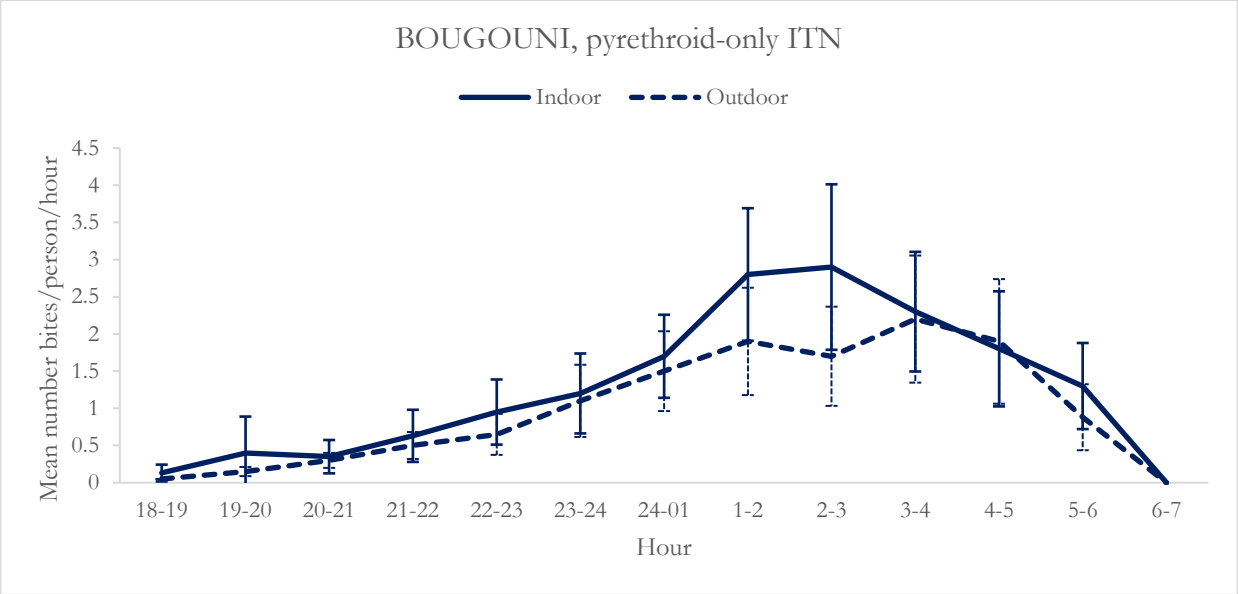
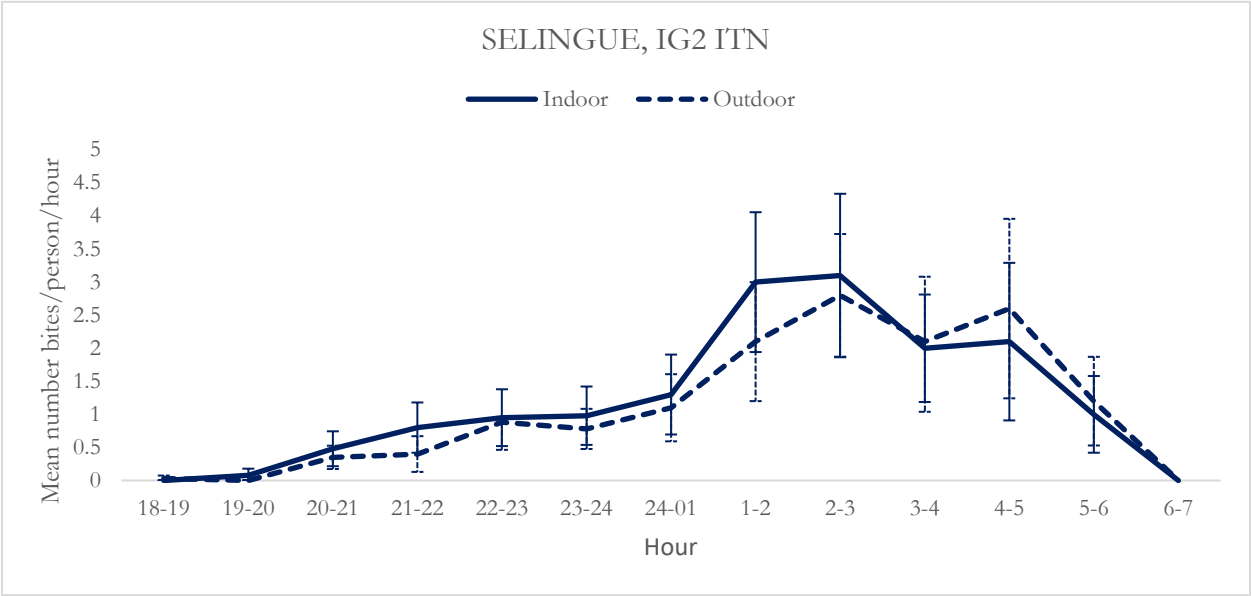


Figure 12: Mean *An. gambiae* s.l. Hourly Biting Rates in Selingue (Interceptor G2 ITN)



3.6 PLASMODIUM SPOROZOITE RATE

Sporozoite ELISA results for *An. gambiae* s.l. collected by HLC are shown in Table 2. No infected vector (0.0% sporozoite rate) was recorded in both sites (sprayed and unsprayed) in Bandiagara during the survey. In Djenné district, 1.6% (5/301) of vectors were infected in Madiama (sprayed) and 0.6% (3/461) in Soala (unsprayed). In Mopti, the sporozoite rate was 2.7% (2/74) in the unsprayed site of Sarema and 0.3% (1/333) in the sprayed site of Soufouroulaye. In Sikasso Region, the sporozoite rate was relatively higher in Bougouni (pyrethroid ITN) with 2.0% (11/549) than in Selingue (Interceptor G2 ITN) with 1.3% (6/464).

Table 2: *Anopheles gambiae* s.l. *P. falciparum* sporozoite rate per site from July to December 2020 in Mopti Region and from July to November in the ITN sites (Sikasso Region)

Site, District (2020 Intervention)	Collection Method	Total *An. gambiae* s.l. tested	% Mean sporozoite rate (positive/ tested)
Bendieli, Bandiagara (Fludora fusion SB-WP)	CDC-LT (CBS)	34	0.0% (0/34)
Dandoly, Bandiagara (Unsprayed)	CDC-LT (CBS)	36	0.0% (0/36)
Madiama, Djenné (SumiShield 50WG)	CDC-LT (CBS)	301	1.6% (5/301)
Soala, Djenné (Unsprayed)	CDC-LT (CBS)	461	0.6% (3/461)
Sarema, Mopti (Unsprayed)	CDC-LT (CBS)	74	2.7% (2/74)
Soufouroulaye, Mopti (Actellic 300 CS)	CDC-LT (CBS)	333	0.3% (1/333)
Selingue (IG2 ITN)	HLC	464	1.3% (6/464)
Bougouni (Pyrethroids ITN)	HLC	549	2.0% (11/549)

3.7 ENTOMOLOGICAL INOCULATION RATE (EIR)

The mean entomological inoculation rate (EIR) was calculated by multiplying the mean HBR from HLC or CDC LT per night by the mean sporozoite rate. The EIRs of the data collection period of each site (either May to December = 224 days for Mopti region or July to November = 153 days for Sikasso region) was expressed as the product of the EIR per night and the number of nights during the collection period. Results are presented in Table 3 for the six sites in the IRS districts and in Table 4 for the two ITN sites in Sikasso Region. The EIR was zero ib/p/n in both sites of Bandiagara. In Djenné, it was higher in the sprayed site of Madiama with 13.6 ib/p/cp than in Soala (unsprayed) with 3.8 ib/p/cp. In Mopti also the highest EIR was observed in the sprayed site of Souforoulaye with 11.6 ib/p/cp versus 1.7 ib/p/cp in the unsprayed site of Sarema. The highest EIRs were in the pyrethroid ITN site of Bougouni (44.4 infective bites per person /collection period (ib/p/cp)), followed by Selingue with 29.4 ib/p/cp.

Table 3: *An. gambiae* s.l. indoor entomological inoculation rate for 8 months (May to December 2021) in six sites in the IRS districts in Mopti Region.

	Dandoly, Bandiagara (unsprayed)	Bemdieli Bandiagara (Fludora Fusion)	Sarema, Mopti (unsprayed)	Soufouroulay, Mopti (Actellic)	Soala Djenné (unsprayed)	Madiama, Djenné (SumiShield)
Mean indoor HBR	1.92	0.34	0.49	8.74	5.73	2.28
Sporozoite rate	0.0	0.0	0.016	0.006	0.003	0.27
EIR per night	0.000	0.000	0.007	0.052	0.017	0.061
EIR per collection period (May to December 2021) (224 nights)	0	0	1.7	11.6	3.8	13.6

Table 4: *An. gambiae* s.l. indoor entomological inoculation rate (July to November 2021) for two sites in Sikasso Region.

	Selingue (Interceptor G2)	Bougouni (Pyrethroid net)
Mean indoor HBR per night	14.88	14.51
Sporozoite rate	0.0129	0.02
EIR per night	0.192	0.290
EIR per collection period (July to November 2021) (153 nights)	29.38	44.40

3.8 Residual Efficacy of Insecticide

3.8.1 IRS Quality Assessment and Residual Efficacy Monitoring

WHO cone bioassays on sprayed walls at T0 (within a week of the start of spraying) yielded 100% mortality using susceptible *An. coluzzii* Ngousso strain when exposed to Actellic 300CS, SumiShield 50WG and Fludora Fusion WP-SB on all wall surfaces (mud, painted mud, cement, and painted cement) tested at all sites (Figures 13-16).

Eight months after IRS in Djenné (sprayed with SumiShield 50WG) and Bandiagara (sprayed with Fludora fusion WP-SB) the 5-day mortality was above 90% (Figure 13) and 100% (Figure 14) respectively, on all the type of walls tested. In Bandiagara, a wild pyrethroid-resistant *An. gambiae* s.l. strain was tested from August to October (T2 to T4) when larval collection was possible. The test could not go beyond T4 due to the dry season and dry larval habitats (Figure 15).

In Mopti (sprayed with Actellic 300CS), the 24-hour mortality rate remained above 80% six months post-IRS on all types of surfaces sprayed, except at T6 on cement surfaces where only 72.7% mortality was achieved. The mortality rate dropped below 80% from T7 respectively on mud surfaces with 63.2% and on painted cement surfaces with 75.5% (Figure 16)

Figure 13: Percentage Mortality up to Day 5 through Cone Bioassay with Insectary Reared *An. coluzzii* (Ngousso strain) Tested on Walls Sprayed with SumiShield 50WG in Djenné (Mopti Region)

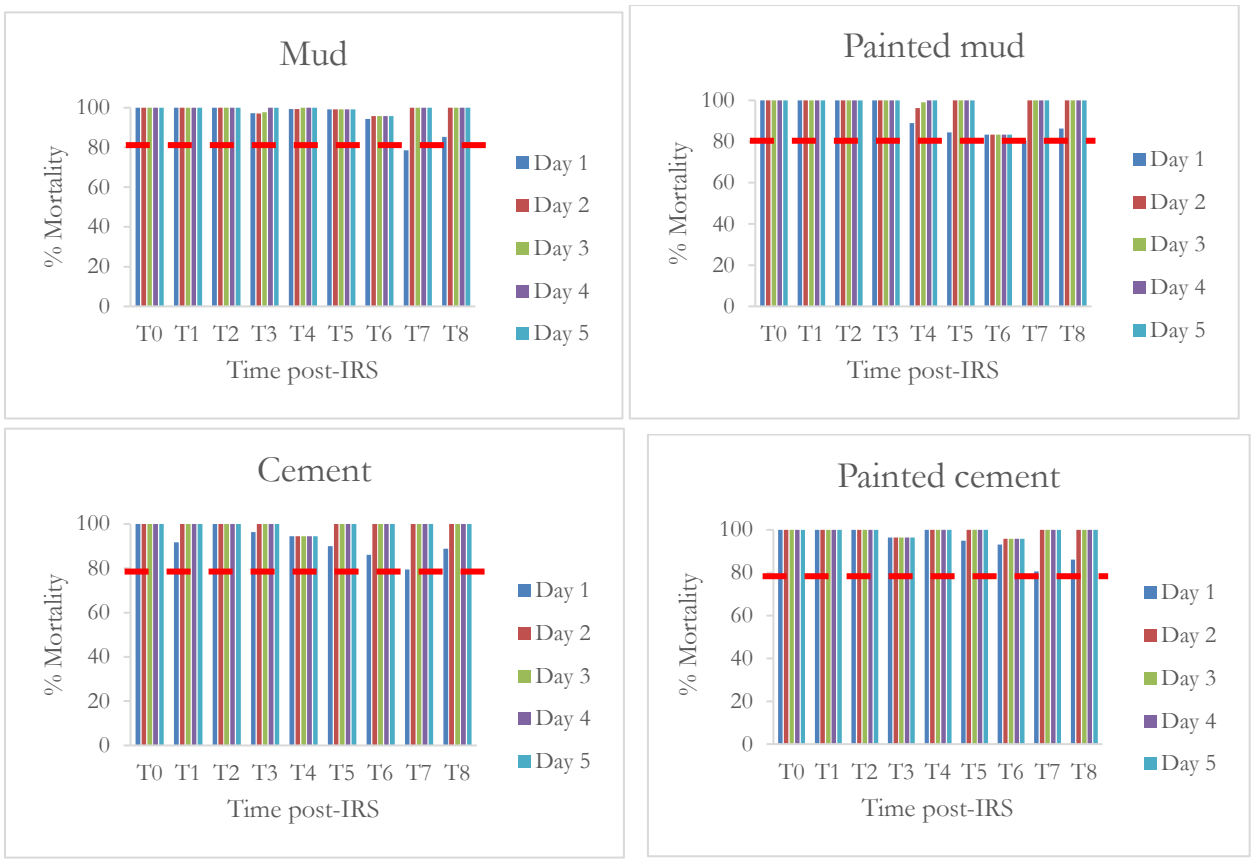
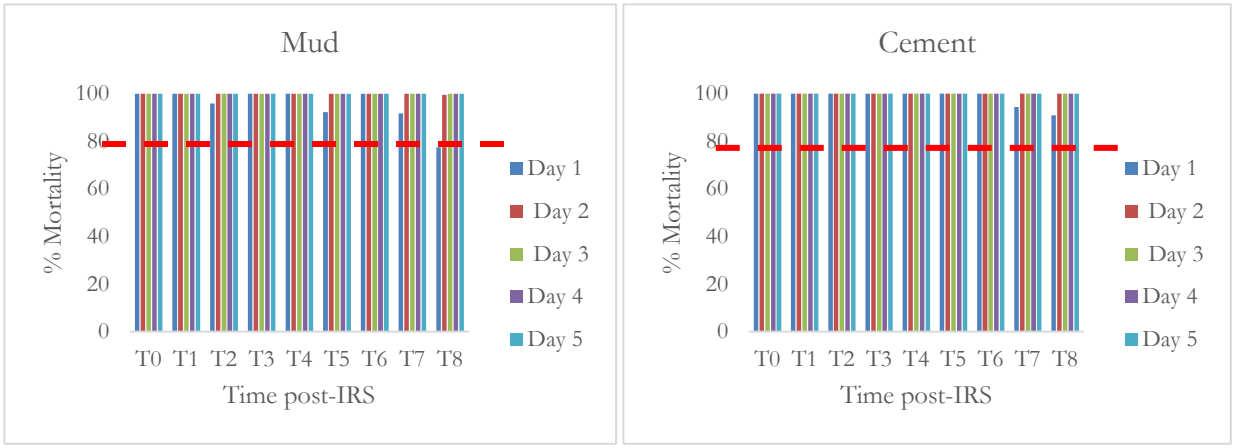


Figure 14: Percentage Mortality up to Day 5 through Cone Bioassay with Insectary Reared *An. coluzzii* (Ngousso strain) Tested on Walls Sprayed with Fludora Fusion WP-SB in Bandiagara



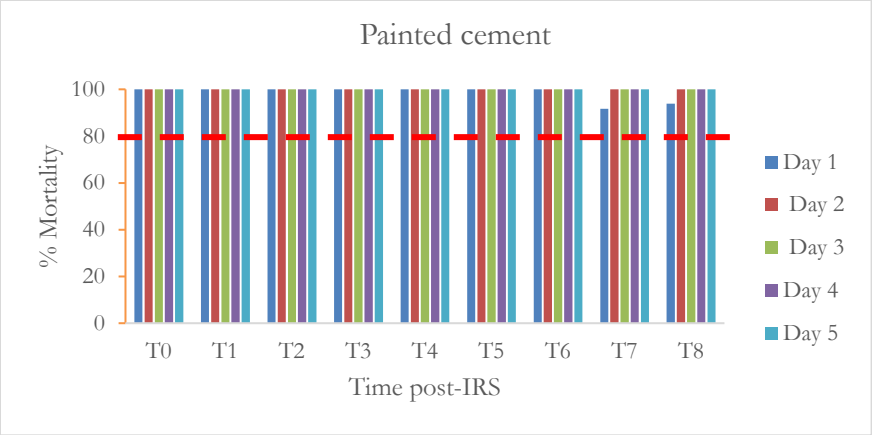
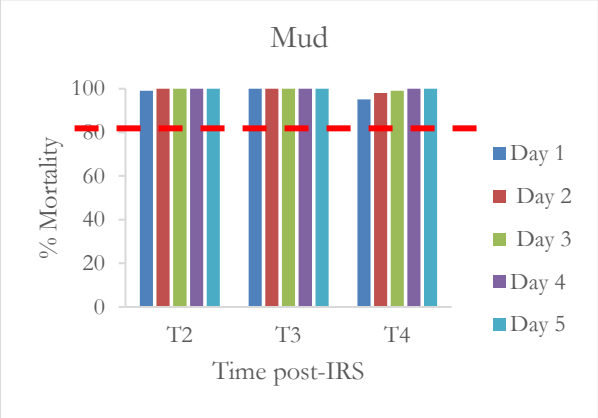
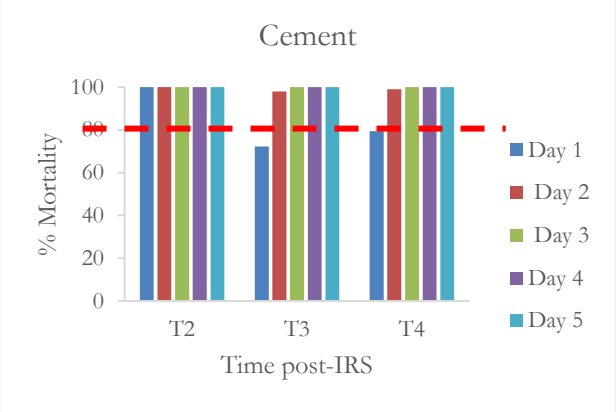


Figure 15: Percentage Mortality up to Day 5 after Cone Bioassay with Wild *An. gambiae* s.l. (reared from larval collection) Tested on Walls Sprayed with Fludora Fusion WP-SB in Bandiagara District (Mopti Region)





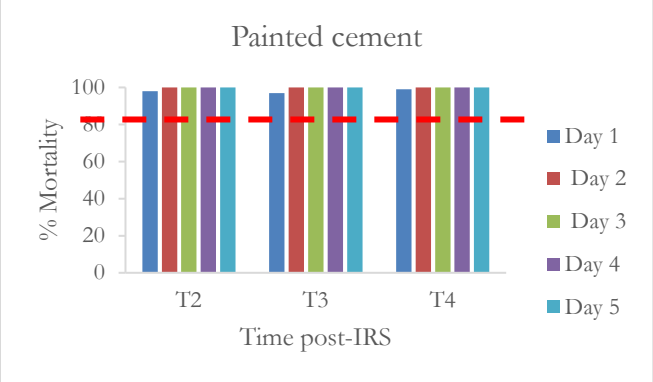
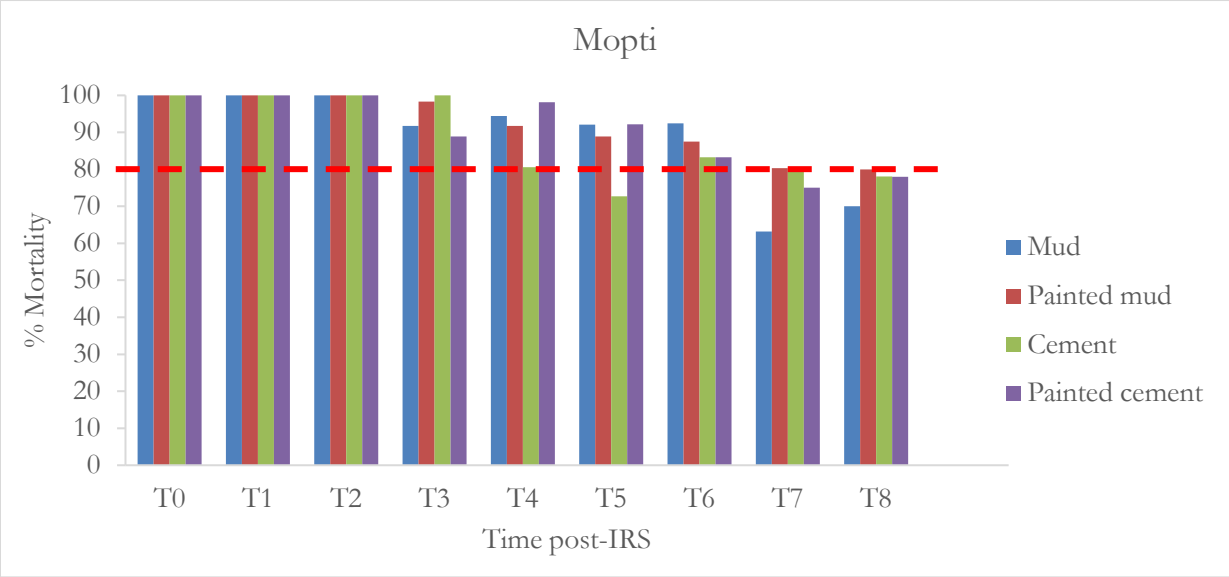


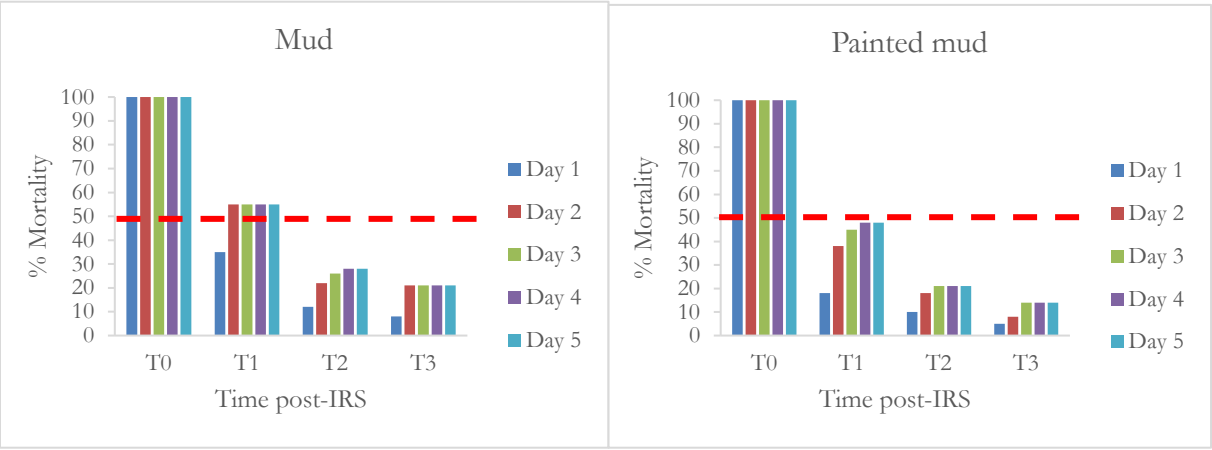
Figure 16: 24-hour Mortality after Cone Bioassay with Insectary Reared *An. coluzzii* (Ngousso strain) Tested on Walls Sprayed with Actellic 300CS in Mopti District



3.8.2 FUMIGANT EFFECT

One hundred percent (100%) mortality was recorded in all sites and to all three insecticides sprayed and all wall types within the first week of spray. The fumigant effect was monitored during three consecutive months after IRS in all sites (Figures 17, 18 and 19). The mortality due to fumigant effect was below the 50% cut-off at the second month in Bandiagara (Fludora Fusion WP-SB) and Djenne (SumiShield 50WG) (Figure 17 & 18). The fumigant effect with Actellic 300CS was, in contrast, below 50% mortality within the first month in Mopti (Figure 19).

Figure 17: Results of fumigant bioassays with insectary reared *An. coluzzii* (Ngousso strain) in Djenné (SumiShield 50wg)



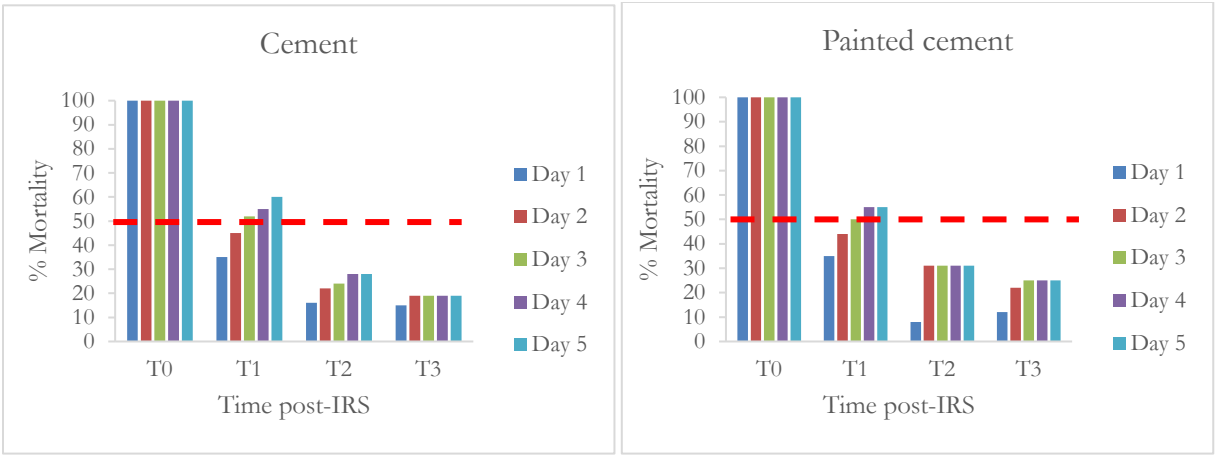


Figure 18: Results of fumigant bioassays with insectary reared *An. coluzzii* (Ngousso strain) in Bandiagara (Fludora Fusion WP-SB)

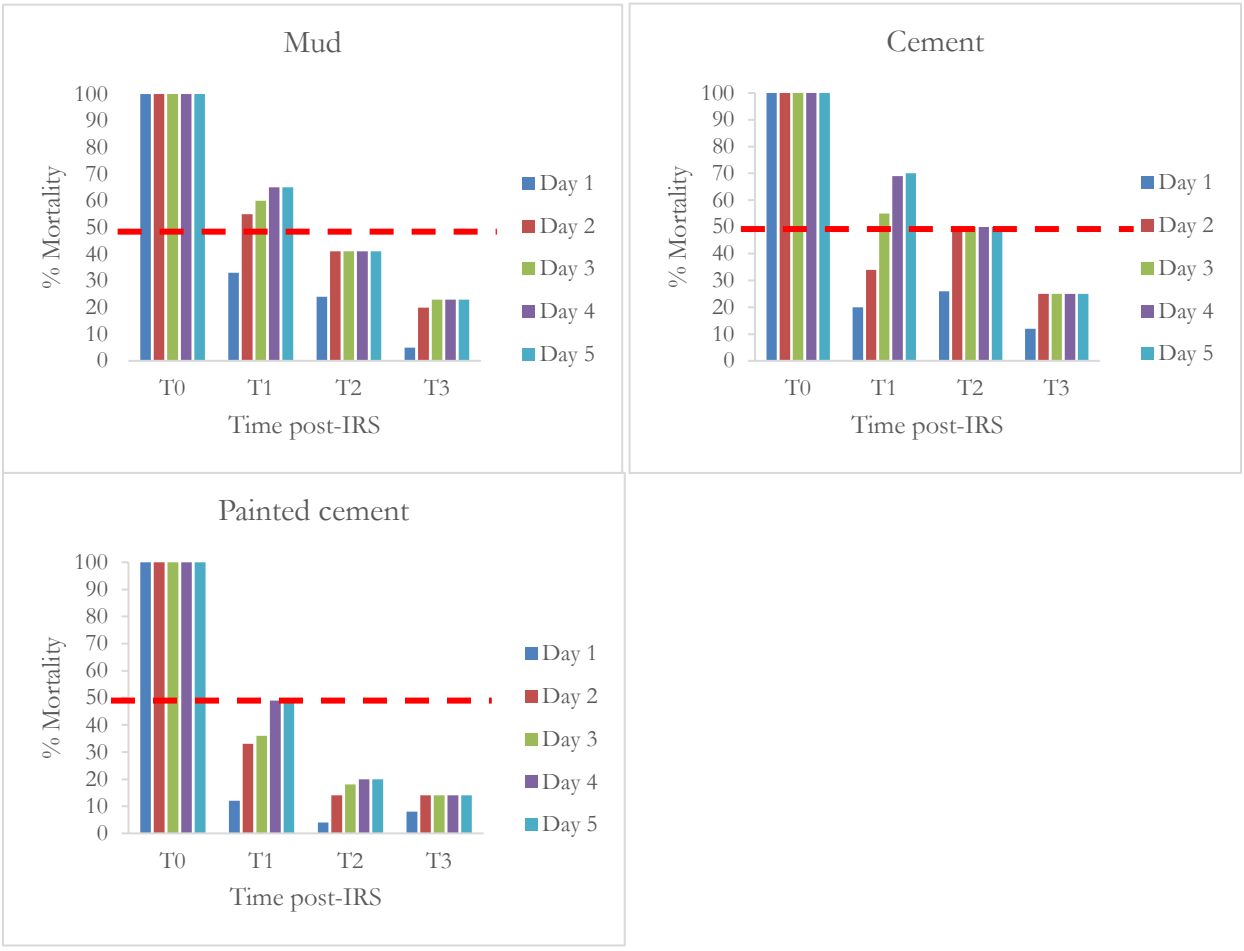
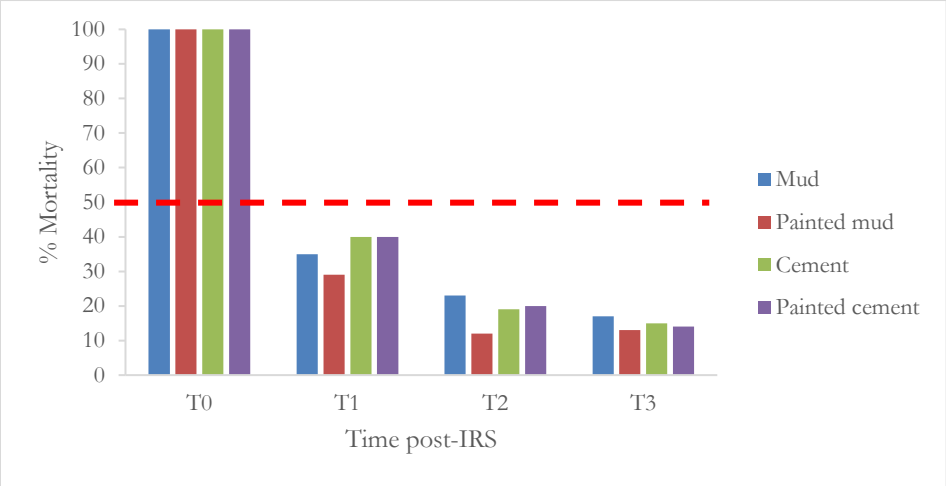


Figure 19: Results of fumigant bioassays with insectary reared *An. coluzzii* (Ngousso strain) in Mopti (Actellic 300CS)



3.9 INSECTICIDE RESISTANCE MONITORING

3.9.1 PYRETHROID RESISTANCE MONITORING

Eight sites were scheduled for insecticide resistance monitoring in 2021. The tests were not performed in Niono due to insecurity between August and November.

Figure 20 shows the percentage mortality of *An. gambiae* s.l. exposed in tube tests to permethrin, deltamethrin and alpha-cypermethrin with and without pre-exposure to PBO. *Anopheles gambiae* s.l. was resistant to the three pyrethroid insecticides (permethrin 0.75%, deltamethrin 0.05%, and alpha-cypermethrin 0.05%) in all seven sites.

Pre-exposure to PBO generally resulted in increased mortality, with variable impact per site and insecticides. While full susceptibility was not restored for any pyrethroid in any site, there was a substantial increase in mortality in four sites (Kita, Bougouni, Djenne and Mopti) when testing with PBO + permethrin compared to permethrin only, all seven sites for PBO + deltamethrin compared to deltamethrin only, and five sites (Kita, Bougouni, Djenne, Selingue and Mopti) for PBO + alpha-cypermethrin compared to alpha-cypermethrin only (Figure 20).

Figure 20: Percentage Mortality 24h of *An. gambiae* s.l. tested with pyrethroids and PBO Synergism

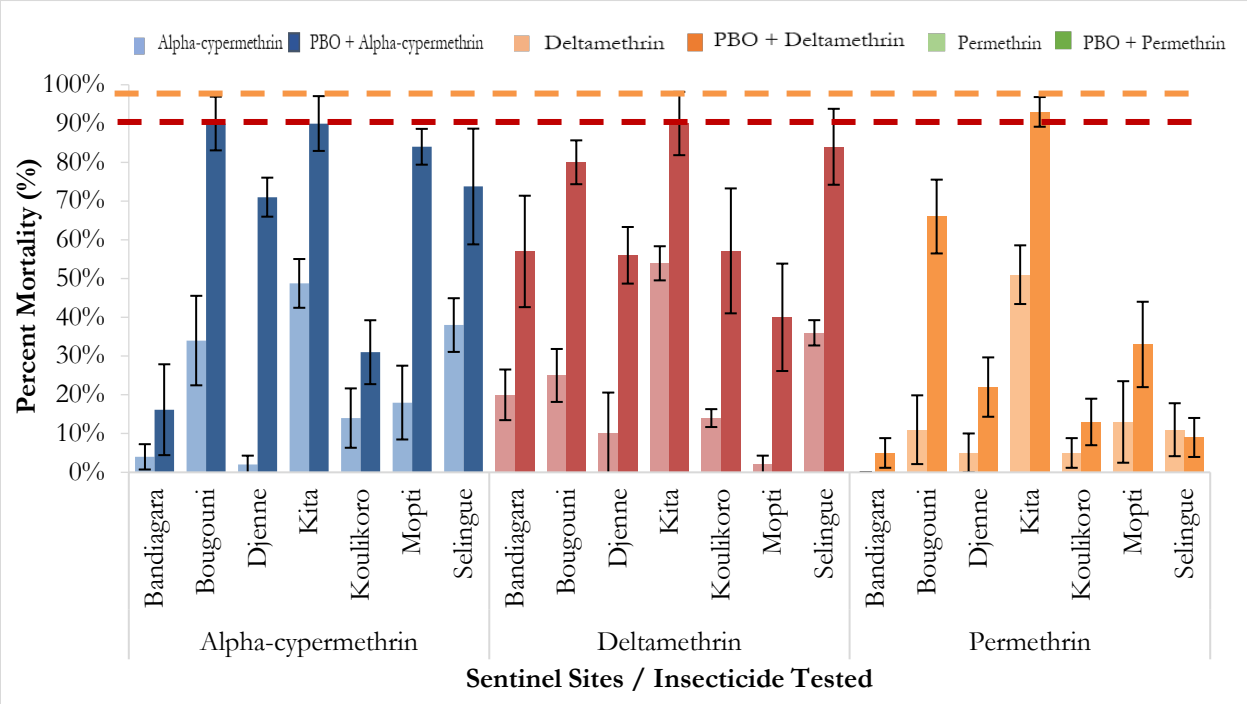
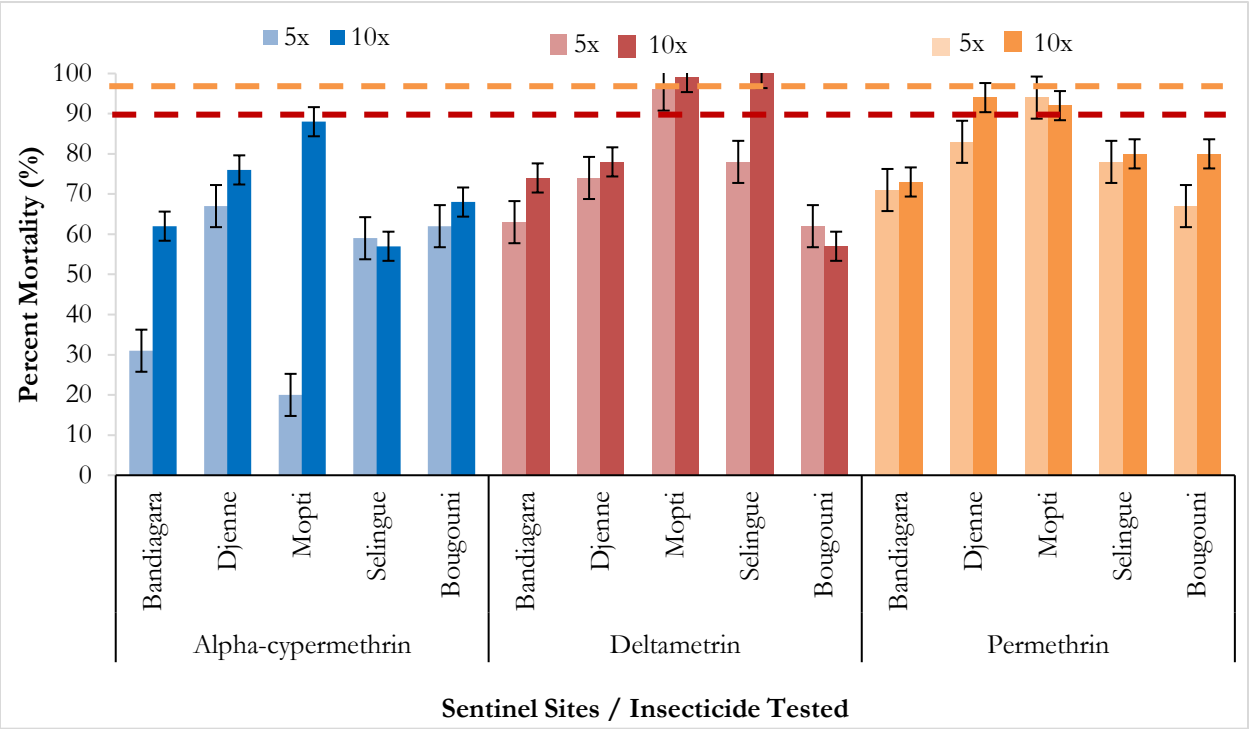


Figure 21 shows resistance intensity data from seven sites against permethrin, deltamethrin and alpha-cypermethrin at one, five and ten times the WHO diagnostic concentration. A high intensity resistance to permethrin and alpha-cypermethrin (mortality <98% at 10× dose) was observed in all seven sites. After exposure to 10x the diagnostic concentration of deltamethrin (0.50%), *An. gambiae* s.l. populations showed a mortality above 98% in Mopti (99%) and Selingue (100%), which is defined as moderate resistance intensity (Figure 21).

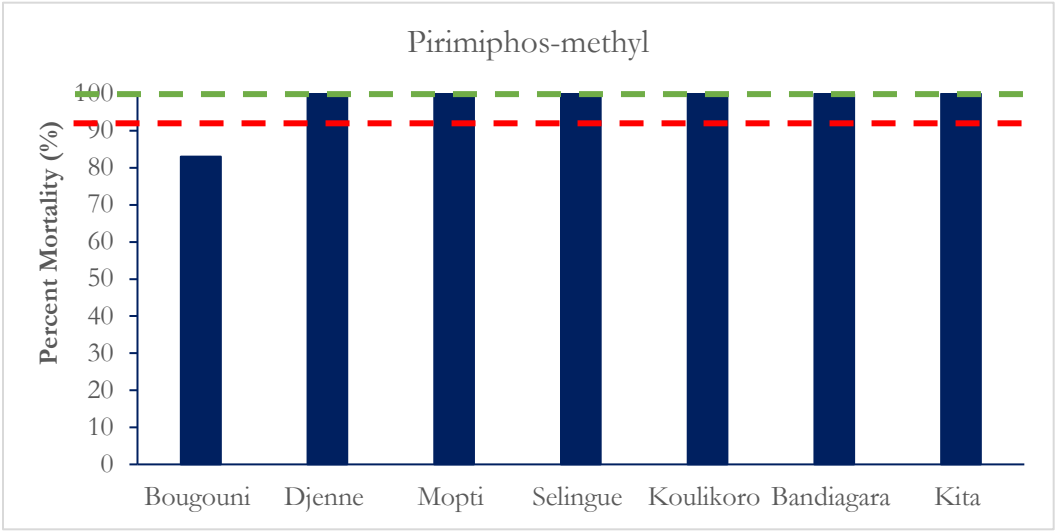
Figure 21: Pyrethroid Resistance Intensity of *An. gambiae* s.l.



3.9.2 PIRIMIPHOS-METHYL RESISTANCE MONITORING

In 2021, susceptibility to pirimiphos-methyl was recorded in six of the seven sentinel sites monitored. Only the site of Bougouni reported resistance to pirimiphos-methyl 0.25% (83.0% mortality) (Figure 22).

Figure 22: Percentage Mortality of *An. gambiae* s.l. Tested against Pirimiphos-Methyl 0.25%

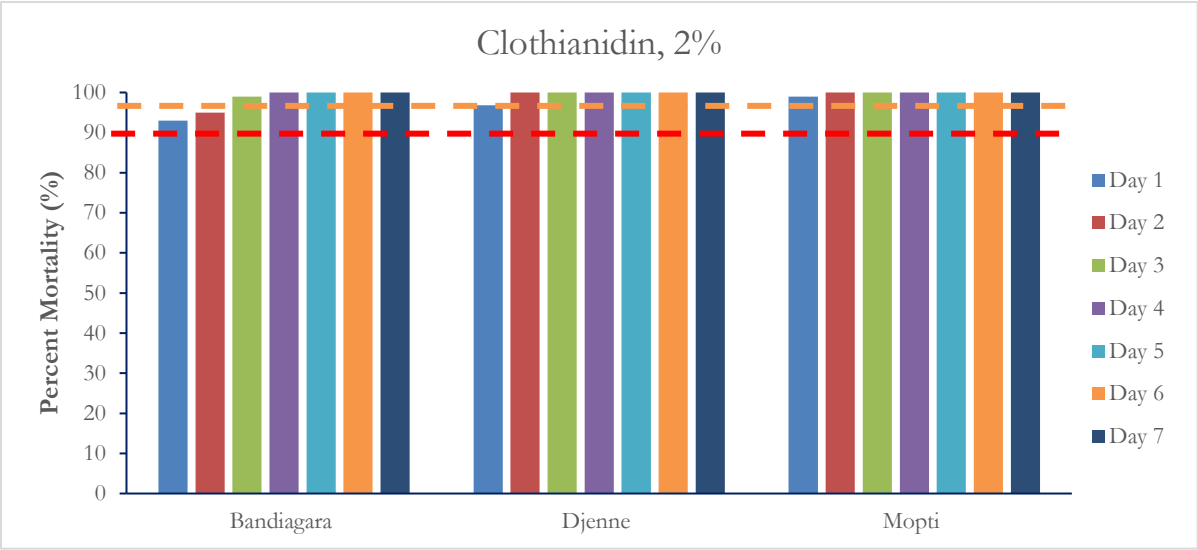


3.9.3 CLOTHIANIDIN RESISTANCE MONITORING

The clothianidin test was prioritized in the IRS districts only.

Figure 23 shows the mortality rates of wild *An. gambiae* s.l. populations following exposure to clothianidin 2%. *An. gambiae* s.l. were susceptible to clothianidin in all three IRS districts (Figure 23).

Figure 23: Percentage Mortality of *An. gambiae* s.l. Tested against Clothianidin 2%

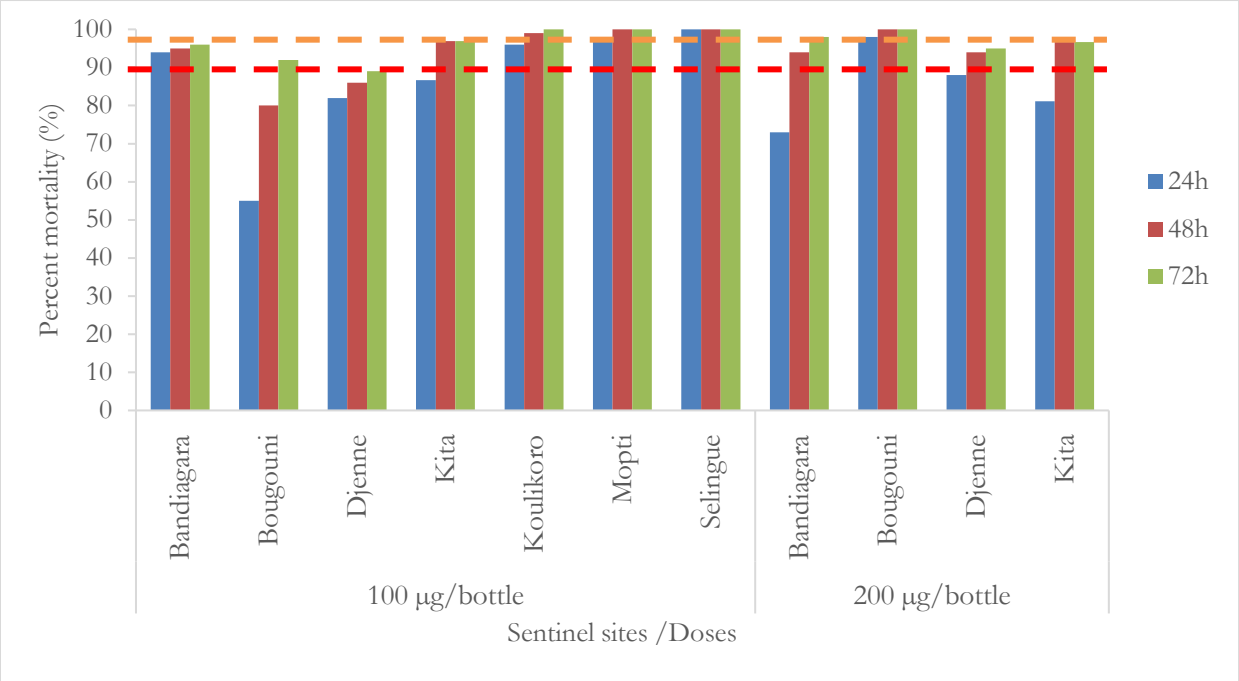


3.9.4 CHLORFENAPYR RESISTANCE MONITORING

Two doses of chlorfenapyr (100µg/bottle and 200µg/bottle) were tested. Figures 24 show the mortality rate of wild *An. gambiae* s.l. populations following exposure to chlorfenapyr 100µg/bottle and 200µg/bottle. An insectary strain of *An. coluzzii* (Ngousso strain) was always tested in parallel to show whether mortality below 98% in the wild population was due to resistance or due to other factors such as test conditions.

For the 100 µg/bottle dose, susceptibility was recorded (mortality was above 98%) for wild *An. gambiae* s.l. at day 3 in three out of seven sites (Koulikoro, Mopti and Selingue), possible resistance was recorded in three sites (Bandiagara, Bougouni and Kita) and resistance in Djenne with 89% mortality (Figure 24). The wild *An. gambiae* s.l showed susceptibility to chlorfenapyr at the dose of 200 µg/bottle in three of the four sites (Bandiagara, Bougouni and Kita) that could not reach susceptibility at the dose of 100 µg/bottle and possible resistance in Djenne (Figure 24).

Figure 24: Percentage Mortality (72h) of Wild *An. gambiae* s.l. Tested against Chlorfenapyr 100 and 200 µg/bottle using CDC Bottle Bioassays



3.10 Molecular Results

The molecular analysis could not be completed fully due to delivery delay of reagents ordered by VectorLink for the subcontracted laboratory. The results from PCR species identification, PCR Kdr-west and Kdr-east and Ace1 will be shared when available through an addendum report

4. Discussion and Conclusion

Longitudinal collection conducted from June to December using CDC LT and PSC or HLC yielded four *Anopheles* species (*An. gambiae* s.l, *An. funestus*, *An. pharoensis* and *An. rufipes*) in the IRS areas. In the two ITN sites, three *Anopheles* species (*An. gambiae* s.l, *An. pharoensis* and *An. rufipes*) were collected during the monitoring period using HLC and PSC. Of all the *Anopheles* collected, 98% were *An. gambiae* s.l. across all sites and all methods. The same mosquito population and diversity was recorded during the previous year's entomological monitoring in the country. Similar to the IRS sites, the collections in the Sikasso sites, where ITNs were distributed in 2020, recorded mainly *An. gambiae* s.l. (96.2%). However, *An. funestus* was not found at all in Sikasso, which could be an important consideration for selecting an appropriate vector control tool in areas with a single malaria vector.

The peak indoor resting densities occurred generally in September in most of the six IRS sites (sprayed and unsprayed). However, the peak IRD was recorded in July in the sprayed site of Bandiagara. The delayed start of the IRS campaign (due to delayed Fludora Fusion delivered) could be the reason, as the density dropped one month after the IRS operations with no subsequent peak recorded. Furthermore, an unexpected higher density was recorded in the IRS site of Mopti when compared to the unsprayed site. The trends observed will require deep quality assessment and data validation of the CBS data collected. Additional training of the collectors, and engagement and communication will need to be conducted to ensure the data reported is of high quality. Also, movement of already trained CBS collectors could be another challenge for increasing experience of collector as the supervisors are mostly government employees. For example, when compared to the 2020 data, more than 5,000 *An. gambiae* s.l were collected at Sarema in 2020, while less than 50 mosquitoes were reported in 2021. This may be due to the change of the community collectors between both years and suggest that density recorded in the unsprayed site of Mopti in 2021 may have been underestimated. It is important for the sustainability to the CBS strategy to have financial motivation for the community collectors.

In the ITN distribution sites, the peak IRD was observed in September in Bougouni and in October in Selingue and the hourly HBR peaked between 1:00 am and3.00 am. As observed last year, the number of *Anopheles* collected by HLC was higher than those collected by PSC and the densities were similar indoors and outdoors.

The highest mean entomological inoculation rate (EIR) was recorded in Bougouni with 0.29 infective bites per person per night (ib/p/n) during the collection period from June to November 2021), followed by Seligue with 0.19 ib/p/n while no infected mosquito was found in the selected samples of both sentinel sites in Bandiagara (unsprayed and sprayed with Fludora Fusion). In Djenné (unsprayed) and Madiana SumiShield sprayed districts, the annual EIR was overall relatively low with 0.02 ib/p/n and 0.06 ib/p/n respectively, over the collection period from May to December 2021 than in Soala (unsprayed) with 3.8 infective bites per person. In Mopti Region, the highest EIR was observed in the sprayed site of Souforoulaye with 0.05 ib/p/n compared to unsprayed site of Sarema (0.01 ib/p/n). While a concrete conclusion could not be drawn from the trends observed in each sprayed district, the lower EIR recorded in Selingue compared to Bougouni where different types of ITNs were distributed may be a positive impact of the ITN type in Selingue. However, the collection methods used in all the sites monitored could also be incriminated. Further data may need to be collected to confirm the trends observed for concrete decision making.

The assessment of IRS efficacy using cone bioassays demonstrated that IRS was sufficiently dosed, with 100% mortality recorded on all types of walls and for all insecticide formulations sprayed within the first week. Furthermore, SumiShield 50WG and Fludora Fusion WP-SB formulations remained efficacious for at least eight months. Actellic 300CS provided a residual efficacy of six months after IRS. These data are similar to those reported last year in Mali and across other VectorLink project IRS countries. However, the fumigant data showed only one month effect for all three insecticides tested and types of walls, which contrasts with

data recorded in a few other countries where longer time of fumigant effect was reported. Additionally, no difference was observed with either surface and surface painted for both residual efficacy and fumigant effect, suggesting that testing a variety of surface types for cone bioassay testing may not be necessary.

Anopheles gambiae s.l. was resistant to the three pyrethroid insecticides (permethrin 0.75%, deltamethrin 0.05%, and alpha-cypermethrin 0.05%) in all sites. Pre-exposure to PBO generally resulted in substantial increased mortality suggesting the involvement of oxidase enzymes in the resistance of the vectors. The PBO effect was variable by site and pyrethroid. The results suggest that PBO + permethrin nets may provide limited benefit over standard pyrethroid nets in some districts (Bandiagara, Selingue and Koulikoro), but could have greater protective potential if used in the district of Kita where higher mortality was observed. PBO + deltamethrin nets could be used in the districts of Kita, Bougouni and Selingue and PBO + alpha-cypermethrin nets could be used Kita, Bougouni, Mopti and Selingue. The resistance intensity tests performed against permethrin, deltamethrin and alpha-cypermethrin showed a high resistance intensity in all sites monitored except in Mopti and Selingue with moderate resistance intensity for deltamethrin. The resistance intensity observed could be a general threat to vector control and may compromise the efficacy of pyrethroid-only ITNs that are being used/distributed in Mali. There is an urgent need to expand the distribution of new generation ITNs in Mali.

Susceptibility to pirimiphos-methyl was found in all sites except in Bougouni where resistance was recorded for the first time. A similar trend was reported in 2020 in the district of Kadiolo where possible resistance to pirimiphos-methyl was recorded. As Bougouni and Kadiaolo belong to two different districts, it is vital to closely monitor pirimiphos-methyl resistance by repeating testing to confirm the trends observed.

Susceptibility to clothianidin was recorded in the three IRS districts, supporting the continued use of clothianidin-based insecticides for IRS.

An. gambiae s.l. was susceptible to chlorfenapyr in Koulikoro, Mopti and Selingue at the dose 100 µg/bottle and in three additional sites at 200 µg/bottle. Only Djenné was still recorded mortality lower than 98%. The test results will need to be repeated in those districts during the next year's susceptibility data collections to confirm the trends observed. However, at this time the results still suggest the use of IG2 nets is appropriate to control the resistant vector population.

ANNEX A

Indoor CDC-LT with attractant										
Districts	**Sites**	**Date / Period**	**June 2021**	**June 2021**	**July 2021**	**August 2021**	**September 2021**	**October 2021**	**November 2021**	**December 2021**
Bandiagara	Bendieli (sprayed)	*An. gambiae s.l.*	0	0	3	14	16	0	1	0
	Dandoli (unsprayed)	*An. gambiae s.l.*	0	0	2	18	8	14	4	0
Djenne	Madiama (sprayed)	*An. gambiae s.l.*	0	1	3	131	172	52	21	12
		An. pharoensis	0	0	0	3	2	1	1	0
	Soala (unsprayed)	*An. gambiae s.l.*	0	0	29	461	177	106	137	62
		An. pharoensis	0	0	0	0	0	1	0	0
Mopti	Soufourou laye (sprayed)	*An. gambiae s.l.*	0	0	8	368	263	55	64	29
		An. pharoensis	0	0	2	1	2	1	8	2
	Sarema (unsprayed)	*An. gambiae s.l.*	1	0	0	35	42	11	22	5
		An. pharoensis	0	0	0	1	0	2	2	0

PSC										
Districts	**Sites**	**Date / Period**	**May 2021**	**June 2021**	**July 2021**	**August 2021**	**September 2021**	**October 2021**	**November 2021**	**December 2021**
Bandiagara	Bendieli (sprayed)	*An. gambiae s.l.*	0	2	30	4	24	3	0	1
	Dandoli (unsprayed)	*An. gambiae s.l.*	1	1	60	133	184	95	20	5
Djenne	Madiama (sprayed)	*An. gambiae s.l.*	0	0	4	85	111	22	25	14
		An. pharoensis	0	0	0	0	0	0	0	0
	Soala (unsprayed)	*An. gambiae s.l.*	2	5	19	152	120	330	31	13
		An. pharoensis	0	1	0	0	0	0	0	0
		An. rufipes	0	0	0	0	0	2	0	0
Mopti	Soufouroulaye (sprayed)	*An. gambiae s.l.*	0	0	4	421	792	355	138	20
		An. pharoensis	0	0	1	0	0	0	0	0
		An. rufipes	0	0	0	0	0	1	0	1
		An. funestus s.l.	0	0	0	0	0	4	0	0
	Sarema (unsprayed)	*An. gambiae s.l.*	0	0	2	7	9	4	1	0
Bougounii	Dalabani (pyrethroid ITN)	*An. gambiae s.l.*	0	0	79	54	155	117	80	0
		An. pharoensis	0	0	1	0	0	0	0	0
Selingue	Gueleba 2 (IG2 ITN)	*An. gambiae s.l.*	0	0	27	30	30	95	10	0

HLC							
Districts	**Sites**	**Date / period**	**July 2021**	**August 2021**	**September 2021**	**October 2021**	**November 2021**
Bougounii	Dalabani (pyrethroid ITN)	*An. gambiae s.l.*	181	414	415	122	28
		An. pharoensis	11	3	3	5	
		An. rufipes	0	0	0	0	0
		An. funestus s.l.	0	2	0	0	0
Selingue	Gueleba 2 (IG2 ITN)	*An. gambiae s.l.*	31	326	566	250	17
		An. pharoensis	9		2	80	
		An. rufipes	0	0	0	0	0
		An. funestus s.l.	0	2	0	0	0

Indoor Resting Density										
Districts	**Sites**	**Date / Period**	**May 2021**	**June 2021**	**July 2021**	**August 2021**	**September 2021**	**October 2021**	**November 2021**	**December 2021**
Bandiagara	Bendieli (sprayed)	*An. gambiae* s.l.	0	0.17	2.5	0.33	2	0.25	0	0.08
	Bendieli (sprayed)	Number of Collections	3	12	12	12	12	12	12	12
	Dandoli (unsprayed)	*An. gambiae* s.l.	0.33	0.08	5	11.1	15.3	7.9	1.7	0.42
	Dandoli (unsprayed)	Number of Collections	3	12	12	12	12	12	12	12
Djenne	Madiama (sprayed)	*An. gambiae* s.l.	0	0	0.33	7.1	9.3	1.8	2.1	1.2
	Madiama (sprayed)	Number of Collections	3	12	12	12	12	12	12	12
	Soala (unsprayed)	*An. gambiae* s.l.	0.67	0.42	1.6	12.7	10	27.5	2.6	1.1
	Soala (unsprayed)	Number of Collections	3	12	12	12	12	12	12	12
Mopti	Soufouroulaye (sprayed)	*An. gambiae* s.l.	0	0	0.33	35.1	66	29.6	11.5	1.7
	Soufouroulaye (sprayed)	Number of Collections	3	12	12	12	12	12	12	12
	Sarema (unsprayed)	*An. gambiae* s.l.	0	0	0.17	0.58	0.75	0.33	0.08	0
	Sarema (unsprayed)	Number of Collections	3	12	12	12	12	12	12	12
Bougouni	Dalabani	An. gambiae s.l.	0	0	4	2.7	7.8	5.9	4	0
	Dalabani	Number of Collections	0	0	20	20	20	20	20	0
Selingue	Gueleba 2	*An. gambiae* s.l.	0	0	1.4	1.5	1.5	4.8	0.5	0
	Gueleba 3	Number of Collections	0	0	20	20	20	20	20	0

	PSC				CDC-LT			
	An. gambiae s.l.	*An. funestus* s.I.	*An. pharoensis*	*An. rufipes*	*An. gambiae* s.l.	*An. funestus* s.I.	*An. pharoensis*	*An. rufipes*
Bandiagara (unsprayed)	499	0	0	0	46	0	0	0
Djenne (sprayed)	261	0	0	0	392	0	7	4
Mopti (unsprayed)	23	0	0	0	116	0	5	0
Bandiagara (sprayed)	64	0	0	0	34	0	0	0
Djenne (unsprayed)	672	0	1	2	972	1	1	0
Mopti (sprayed)	1730	4	1	2	787	6	14	18
Total	**3249**	**4**	**2**	**4**	**2347**	**7**	**27**	**22**