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PMI VECTORLINK PROJECT MALI ANNUAL ENTOMOLOGICAL MONITORING REPORT JANUARY 2020 – DECEMBER 2020

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PMI VECTORLINK MALI ANNUAL ENTOMOLOGICAL MONITORING REPORT JANUARY 2020 - DECEMBER 2020

CONTENTS

LIST OF TABLES

LIST OF FIGURES

ACRONYMS

CS	Capsule suspension
CSP	Circumsporozoite protein
EIR	Entomological inoculation rate
ELISA	Enzyme-linked immunosorbent assay
HBR	Human biting rate
HLC	Human landing catch
IRS	Indoor residual spraying
ITN	Insecticide-treated net
LBMA	*Laboratoire de Biologie Moléculaire Appliquée*, Applied Molecular Biology Laboratory
NMCP	National Malaria Control Program
PBO	Piperonyl butoxide
PMI	President's Malaria Initiative
PSC	Pyrethrum spray catch
WG	Wettable granules

EXECUTIVE SUMMARY

The 2020 Mali indoor residual spraying (IRS) campaign was implemented from June 15th, 2020 to July 24th, 2020, in the districts of Bandiagara and Djenné. Due to delays in the Fludora® Fusion WP-SB shipment caused by international supply chain disruptions related to the COVID-19 pandemic, the 11 operations sites in Mopti District started on June 29, two weeks later than scheduled and ended August 12th, 2020. Actellic® 300CS (capsule suspension) was sprayed in Djenné District, SumiShield® 50WG in Bandiagara District and Fludora® Fusion WP-SB in Mopti District.

To determine insecticide residual efficacy cone bioassays were conducted monthly on sprayed walls in each of the three IRS districts (Mopti, Bandiagara and Djenné). Entomological surveillance of *Anopheles* malaria vector species was conducted in three sprayed sites (Mopti, Bandiagara and Djenné) and a neighboring unsprayed site (Tominian) using pyrethrum spray catch (PSC) and human landing catch (HLC). Community-based surveillance (CBS) was also piloted from August 2020 in two sites in Mopti district (one sprayed, one unsprayed) using CDC light traps set indoors. To monitor the impact of the Interceptor® G2 insecticide-treated nets (ITNs) which were distributed in June 2020, VectorLink Mali conducted longitudinal monitoring through HLC and PSC in two sites in Sikasso Region: Selingué where Interceptor® G2 nets were distributed and a control site in Bougouni where pyrethroid-only ITNs were distributed. Insecticide susceptibility tests were conducted in 10 sites to inform the choice of insecticide for future IRS and ITN campaigns.

Cone bioassays demonstrated that IRS was satisfactory, with 100% mortality recorded on all types of walls and for all insecticide formulations sprayed. All three insecticide formulations produced 100% mortality of an insectary strain of *An. coluzzii* for at least seven months (testing will continue until mortality falls below 80% for two consecutive months). In Mopti (Fludora Fusion WP-SB) cone bioassay also was efficacious against wild pyrethroid resistant *An. gambiae* s.l. up to four months after spraying (it was not possible to collect wild mosquitoes for bioassay after four months due to the dry season). According to these results the three insecticides (Actellic 300CS, SumiShield 50WG and Fludora Fusion WP-SB) provide control for the duration of the peak malaria transmission season and can be used in the next IRS campaign in Mopti Region as part of a rotation strategy to mitigate the development of resistance.

The most common *Anopheles* species collected by HLC, PSC and CDC LT was *An. gambiae* s.l. in all sites (96-100% at each site). Molecular analysis showed that *An. coluzzii* was the predominant species in Mopti, Djenné, Bandiagara (in Mopti Region), Tominian (Segou Region) and Selingue (Sikasso Region), while *An. gambiae* was the predominant species in Bougouni (Sikasso Region). The peak indoor resting densities and human biting rates occurred in August and September in all sites in Mopti and Segou regions, while in Sikasso Region the peak transmission season lasted from August to October.

Over the six-month monitoring period the mean indoor resting density of *An. gambiae* s.l. was 4.1 per house per day in Djenné (Actellic 300CS), 5.1 in Bandiagara (SumiShield 50WG), 20.7 in Mopti (Fludora Fusion WP-SB) and highest in unsprayed Tominian with 49.8 *An. gambiae* s.l. per house per day. Biting rate data was affected by insecurity which prevented nighttime supervison in Mopti Region. The community-based surveillance pilot showed no clear impact of spraying on biting rates, although this may have been affected by the delayed IRS campaign in Mopti District.

The most sensitive measure of vector control impact is the malaria sporozoite rate. The mean sporozoite rate was higher in the unsprayed control site of Tominian with 3.73% (28/750) compared to the neighboring paired site of Djenné (Actellic 300CS) with 2.21% (5/226). The sporozoite rate was 0.28% (2/723) in Mopti (Fludora Fusion WP-SB) and 2.24% (5/223) in Bandiagara (SumiShield 50WG). In Sikasso Region the sporozoite rate was 2.5% (15/600) in Bougouni (covered by pyrethroid nets distributed in 2020) and 0.86% (5/581) in Selingue (covered by Interceptor G2 ITNs). The entomological inoculation rate (EIR) was calculated by multiplying the biting rate by malaria sporozoite rate to determine the risk of malaria infection for an individual for the six-

month peak malaria transmission season. In Sikasso Region, the EIR was 119 infectious bites per person in Bougouni (pyrethroid ITN) and 52 infectious bites per person in Selingue (Interceptor G2 ITNs). The EIR was higher in the unsprayed sites of Tominian (114 infectious bites per person) compared to neighboring sprayed Djenné (13 infectious bites per person). One of the reasons for this difference could be the impact of IRS. In Mopti District, an EIR of 14.7 infectious bites per person was recorded in Toguel (unsprayed) and 14.4 infectious bites per person in Sarema (sprayed with Fludora Fusion WP-SB) as measured by community-based surveillance. In the IRS sites of Mopti (HLC) and Bandiagara the EIR was relatively low at 8 and 11 infectious bites per person, respectively. The EIR in Selingue (Interceptor G2 nets) was half that of Bougouni (pyrethroid ITNs). This is an encouraging sign but as there was no baseline data collected in 2019, we cannot attribute the difference to the type of net distributed.

According to WHO criteria, *An. gambiae* s.l. were resistant to the three pyrethroid insecticides (permethrin 0.75%, deltamethrin 0.05%, and alpha-cypermethrin 0.05%) tested in 2020 in all ten sites. There was a significant increase in mortality when testing with PBO + permethrin compared to permethrin only in 3 of 10 sites, 7 of 10 for PBO + deltamethrin and 6 of 10 for PBO + alpha-cypermethrin. PBO + permethrin nets are probably not suitable for use in most sites, as mortality only improved to >50% in 1 site (Djenné), with mortality remaining low in all other sites (or no greater than permethrin alone). With PBO, the mean mortality rate across all sites was only 45-60%. Wild *An. gambiae* s.l were susceptible to chlorfenapyr at the dose of 200µg/bottle in 7 out of 9 sites tested. There were two sites with possible resistance (Bougouni 90% and Selingue 96%) where repeat testing is needed in 2021. Given the limited increase in mortality with PBO and high susceptibility to chlorfenapyr in most sites. *An. gambiae* s.l. were susceptible to clothianidin in all sentinel sites surveyed, including IRS sites of Mopti, Bandiagara and Djenné. There was also full susceptibility to pirimiphos-methyl in 9 of 10 sentinel sites (including all IRS sites). Clothianidin and pirimiphos-methyl formulations should continue to be used in rotation for insecticide resistance management in Mali based on their long residual action (at least 7 months) and continued susceptibility of malaria vectors.

I. INTRODUCTION

Malaria vector control in Mali primarily depends on nationwide use of insecticide-treated nets (ITNs) and targeted application of indoor residual spraying (IRS) in high transmission areas. The US President's Malaria Initiative (PMI) supported annual IRS campaigns in Mali since 2008. Since 2017, IRS operations had been relocated from Koulikoro Region in the south of Mali to Mopti Region in central Mali based on the 2015 epidemiological data showing a 60 percent malaria prevalence rate in Mopti Region compared to 30 percent national average.

The primary objective of the VectorLink Mali project in 2020 was 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 2020 Actellic® 300CS was used in Djenné, SumiShield® 50WG in Bandiagara and Fludora® Fusion WP-SB in Mopti. Fludora Fusion WP-SB was used in Mali for the first time in 2020. Over the period of June 15th to August 12th, 2020, the project sprayed a total of 129,302 structures out of 133,426 structures found by spray operators in the targeted areas, accounting for a coverage rate of 96.9%, despite the highly unstable security conditions and ongoing COVID-19 pandemic.

2. METHODOLOGY

2.1 STUDY AREA

Longitudinal vector surveillance using HLC and PSC was conducted in three IRS sites (Mopti, Bandiagara and Djenné) and one unsprayed control site (Tominian), one site in Sikasso Region where Interceptor G2 nets were distributed (Selingue) plus a neighboring site with pyrethroid nets (Bougouni). A community-based surveillance (CBS) pilot was also conducted monthly using CDC light traps in Socoura (sprayed with Fludora Fusion WP-SB) and Toguel (unsprayed) since August 2020.

Insecticide resistance data (including PBO synergist data) was also collected from 3 IRS sites (Mopti, Bandiagara and Djenné), 1 former IRS district (Bla), 4 sites in in Sikasso Region where Interceptor G2 net (IG2 ITN) and Yorkool net were distributed (Kadiolo, Selingue, Bougouni and Yanfolila) and 2 other sites (Kayes and Bamako) chosen in collaboration with the NMCP (Figure 1).

FIGURE 1: MAP OF MALI SHOWING REGIONAL BOUNDARIES AND 2020 ENTOMOLOGICAL SURVEILLANCE SITES

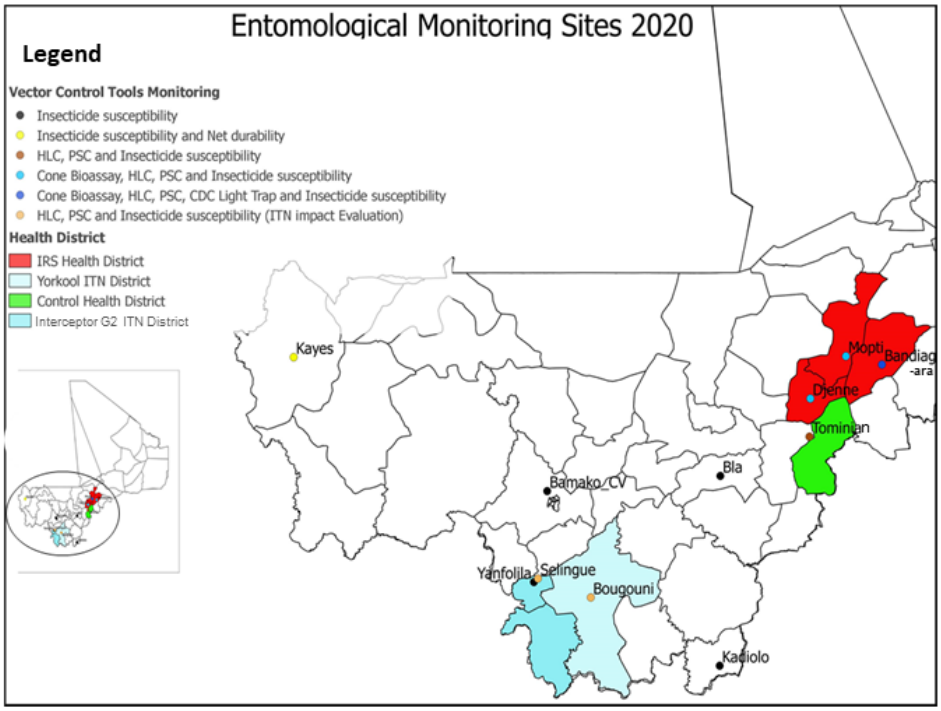


TABLE 1: MONTHLY ENTOMOLOGICAL SURVEILLANCE SITES

Region	District	Health Area	Site (village)	Spray Status	Trapping Period	Trapping Methods	IRS History or ITN Mass Campaign
Mopti	Mopti	Socoura	Sarema	Sprayed	June to December	HLC & PSC	OP 2017, SS 2018, OP 2019, FF 2020. PYR ITN 2020.
	Bandiagara	Bandiagara central	Dandoly	Sprayed			OP 2017, 2018, 2019, SS 2020, PYR ITN 2020
	Djenné	Madiama	Madiama	Sprayed			OP 2017, SS 2018, SS 2019, OP 2020, PYR ITN 2020.
Segou	Tominian	Ouan	Ouena	Unsprayed			Unsprayed 2017-20, PYR ITN 2019.
Sikasso	Selingue	Tinko		Unsprayed	August to December	HLC & PSC	Interceptor G2 Net
	Bougouni	Bougouni sud		Unsprayed			Yorkool Net
Mopti	Mopti	Socuoura	Sarema	Sprayed	August to December	CDC LT	OP 2017, SS 2018, OP 2019, FF 2020, PYR ITN 2020.
			Toguel	Unsprayed			Unsprayed 2017-20, PYR ITN 2020.

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

2.2 LONGITUDINAL MONITORING

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

2.2.1 HUMAN LANDING CATCH

Human landing catches (HLCs) were carried out in each site from 06:00 pm to 08:00 am 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:

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

2.2.2 PYRETHRUM SPRAY CATCH

The ZZ Paff aerosol spray containing permethrin 0.25%, tetramethrin 0.20%, D-phenothrin 0.01% and PBO 0.34% was used to perform pyrethrum spray catch (PSC) in 20 houses per site per month to sample indoor resting mosquitoes. Ten houses per day were surveyed from 7:00 a.m. to 10:00 a.m. during two consecutive

days (twenty houses total per month), with the same houses used every month. All female *Anopheles* were assessed for their abdominal status (unfed, fed, half gravid and gravid) and identified to species using the key of Gillies and Coetzee, 1987. PSC results are presented as the mean indoor resting density per house according to the following calculation:

$$\text{Mean monthly indoor resting density} = \frac{\text{total number of An. gambiae s.l. collected by PSC per month}}{\text{total number of houses surveyed per month}}$$

2.2.3 COMMUNITY-BASED SURVEILLANCE (CBS)

Community-based entomological surveillance using CDC-light trap collections was piloted in 2020 by VectorLink Mali in two sites in Mopti District; one site was sprayed with Fludora® Fusion WP-SB (Sarema) and a non-sprayed village (Toguel). Community-based mosquito collectors received basic training on the use and maintenance of equipment and SOPs on the use of CDC light traps, completion of data forms, and storage of mosquito samples. Community mosquito collectors set 3 traps per night indoors for 20 nights per month (60 houses total per month). The same 60 houses were monitored each month for each site. Community mosquito collectors (one per site) submitted data forms to VectorLink Mali entomology staff during monthly supervision visits. Mosquito samples were stored in the fridge at the local health facility before being collected by VectorLink each month. Community collectors only conducted basic identification as *Anopheles* or Culicine (all Culicines were kept for cross-checking). Morphological identification of samples was subsequently done by experienced VectorLink technicians. Next year we plan to improve the morphological identification skills of community-based collectors so that they can conduct identification to species level. After confirmation of species identification, *An. gambiae* s.l samples were stored individually in Eppendorf tubes with 70% ethanol. A sub-sample was sent to the LBMA for molecular analysis. CDC LTs collect host seeking mosquitoes and is 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:

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

2.3 IRS QUALITY ASSESSMENT AND RESIDUAL EFFICACY MONITORING

World Health Organization (WHO) cone wall bioassays and fumigant bioassays were conducted monthly in the three IRS sites (Mopti, Bandiagara and Djenné). The first quality assessment was done within a week of the IRS campaign starting and bioassays were repeated monthly in the same houses for residual efficacy monitoring. Bioassay results are reported from July through December 2020 (testing will continue in 2021 until mortality is less than 80% for two consecutive months). Cone bioassays were conducted in a total of 30 structures (10 in Djenné sprayed with Actellic 300CS , 10 in Mopti sprayed with Fludora Fusion WP-SB and 10 in Bandiagara sprayed with SumiShield 50WG) with three cones tested per room at heights of 0.5, 1.0, and 1.5m. 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. Mortality rates were recorded 24 hours after exposure for Actellic 300CS and every 24 hours for up to 7 days for SumiShield 50WG and Fludora Fusion WP-SB. Negative controls consisted of at least 30 female *An. coluzzii* tested in parallel on an unsprayed wall in each site (one unsprayed house was identified and used for bioassay).

The contribution of airborne effects to overall mortality in cone bioassays was also assessed using fumigant bioassays according to project SOPs.

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 *Anopheles gambiae*, *An. coluzzii* and *An. arabiensis*. The head and thorax of the same specimens collected through routine monthly 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:

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

2.5 Insecticide Susceptibility Testing

2.5.1 WHO Susceptibility Tests

From June to October 2020, the VectorLink Mali team collected *Anopheles* larvae in 10 sites (Selingue, Yanfolila, Bougouni, Kadiolo, Kayes, Bamako, Bla, Mopti, Djenne and Bandiagara) and reared to adults for insecticide susceptibility tests according to standard project SOPs. Tests were performed with the diagnostic concentration of permethrin (0.75%), alpha-cypermethrin (0.05%), deltamethrin (0.05%) and pirimiphos-methyl (0.25%). Further tube tests were conducted with clothianidin (2% SumiShield filter papers) using wild *An. gambiae* s.l.. All papers (except for clothianidin) were prepared by the WHO collaborating center, University Sains Malaysia. The clothianidin dosage was determined based on internal testing conducted by Sumitomo, which showed 2% weight/volume clothianidin to be a suitable diagnostic concentration for each treated filter paper. Mortality was recorded 24 hours after exposure for all insecticides except clothianidin, which was recorded daily for up to seven days, in order to record any delayed mortality effects. All the clothianidin tests were performed in the VectorLink lab in Bamako with the susceptible *An. coluzzii* strain being tested in parallel as a positive control to ensure papers were dosed correctly.

The result was interprated as follow:

- % mortality between 98% and 100% = Susceptibility
- % moratlity <98% and > 90% = Possible resistance (to be confirmed)
- % mortality < 90% = Resistance

2.5.2 CDC Bottle Bioassays

Chlorfenapyr was tested using CDC bottle bioassays at 100µg (interim diagnostic dose) and 200µg per bottle. Both the susceptible strain and the field mosquitoes were tested at the same time for each site. All chlorfenapyr tests were performed in the VectorLink bioassay room in Bamako to control bioassay conditions. The exposure time was 30 minutes, with mortality recorded daily for up to 72h to record any delayed mortality effects.

2.5.3 PBO Synergist-insecticide Bioassays

Synergist assays were conducted by pre-exposing mosquitoes to WHO papers treated with piperonyl butoxide (4%) for 60 minutes before being immediately transferred to a different WHO tube with a pyrethroid-treated paper for further 60 minutes. After exposure, all mosquitoes were transferred to a holding tube and provided with cotton pads soaked in 10% sugar solution and held for 24 hours. Mortality was scored 24 hours after exposure.

2.5.4 Molecular Characterization of Mosquitoes

Dead and surviving mosquitoes from susceptibility tests were preserved individually in 1.5ml Eppendorf tubes containing 70% ethanol for future molecular analyses (polymerase chain reaction (PCR) species, *kdr* 1014F, *kdr* 1014S and *ace1*R). Identification of *An. gambiae* subspecies was performed according to the protocol described by Santolamazza et al., 2008. The protocol of Huynh et al., (2007) was used to identify the presence of voltage gated sodium channel (Vgsc) L1014F and L1014S mutations within populations of *An. gambiae* s.l. The detection of the *ace-1R* mutation was done following the protocol described by Weill et al. (2004).

2.6 Data Management

The DHIS2-based VectorLink Collect programs for entomological data management were used in Mali for the first time in 2020. The Home Office staff remotely trained and supported Abt entomologists and the database manager on updated data work flows - including field paper collections, technical reviews, data entry, data cleaning, and analytics - to support the generation and use of high quality entomological data. All entomological data collected in Mali in 2020 was managed within VectorLink Collect. The platform includes comprehensive dashboards to synthesize vector bionomics and insecticide resistance summary results. In 2021, stakeholders including NMCP and PMI, will have ongoing access to these results dashboards to support timely decision-making.

3. RESULTS

3.1 MALARIA VECTOR SPECIES COMPOSITION

Over the period of July to December 2020, a total of 12,278 *Anopheles* species were collected by HLC and PSC in the three IRS Districts (Mopti, Djenné and Bandiagara) and one unsprayed control district (Tominian). Four different *Anopheles* species were collected (*An. gambiae* s.l., *An. funestus* s.l., *An. pharoensis*, and *An. rufipes*) during the monitoring period. Approximately 22% (2,712) of *Anopheles* were collected by HLC and 78% (9,566) were collected through PSC (sampling effort was performed by PSC with 20 houses per month and 16 trap nights per month by HLC). *Anopheles gambiae* s.l was the predominant species collected accounting for 99% (12,166) of *Anopheles* collected by HLC and PSC followed by 0.4% (55) *An. pharoensis*, 0.3% (41) *An. rufipes* and 0.1% (16) *An. funestus*. Species composition by HLC and PSC for the four sites combined is shown in Figure 2 and broken down by site in Figure 3. *Anopheles gambiae* s.l. was the predominant species in Bandiagara (98%), Djenné (97%), Mopti (99%) and Tominian (>99%) (Figure 3).

FIGURE 2: SPECIES COMPOSITION OF *ANOPHELES* COLLECTED BY HUMAN LANDING AND PYRETHRUM SPRAY CATCHES IN ALL FOUR IRS RELATED SITES (BANDIAGARA, DJENNÉ, MOPTI, TOMINIAN) FROM JULY TO DECEMBER 2020 (TOTAL *ANOPHELES* COLLECTED = 12,278)

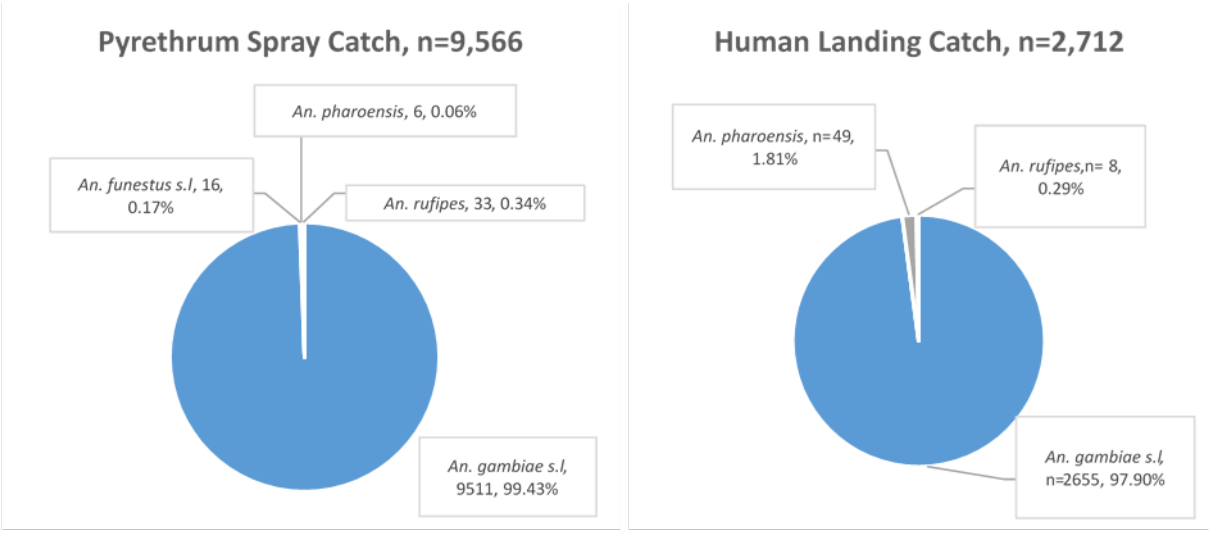
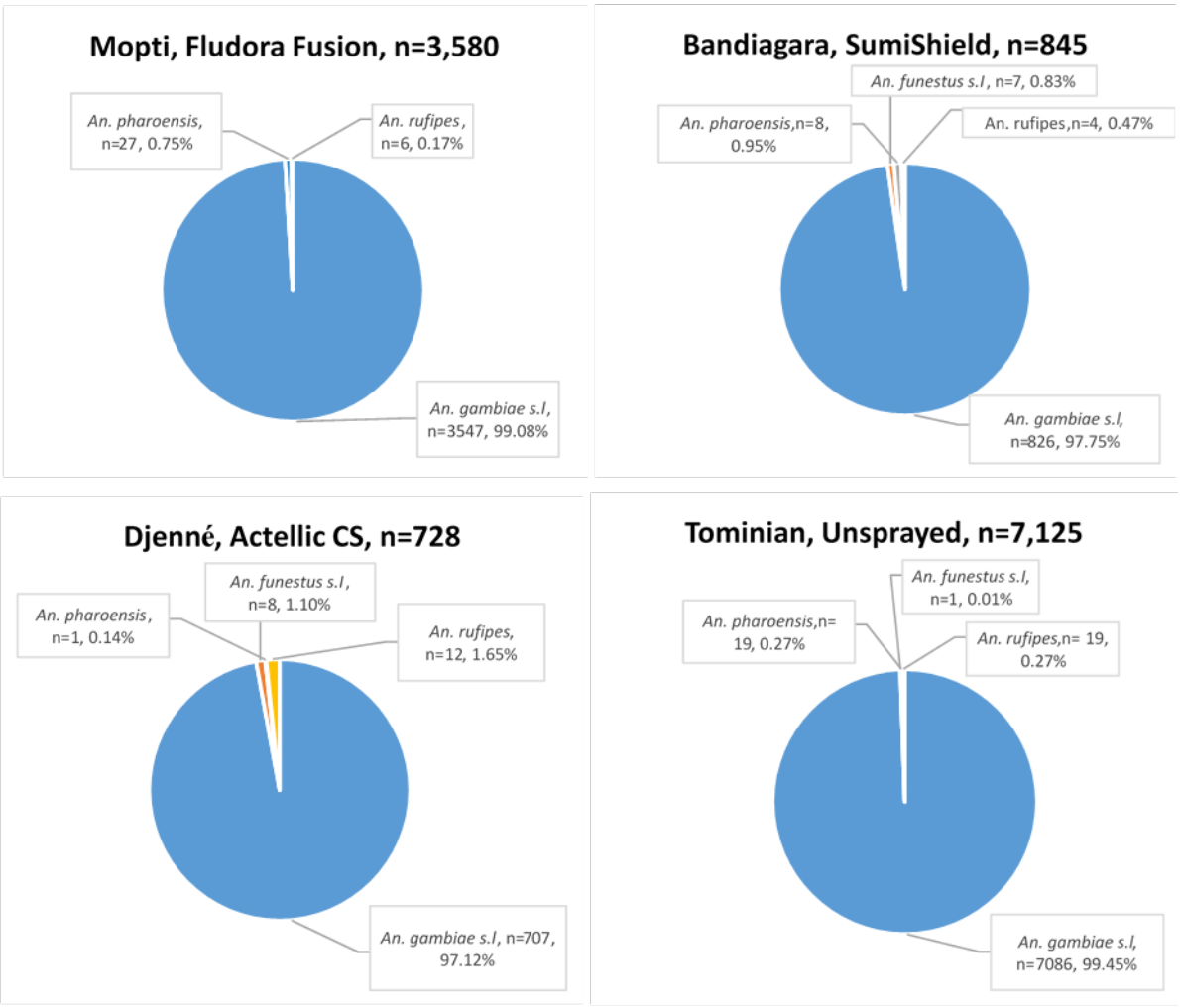
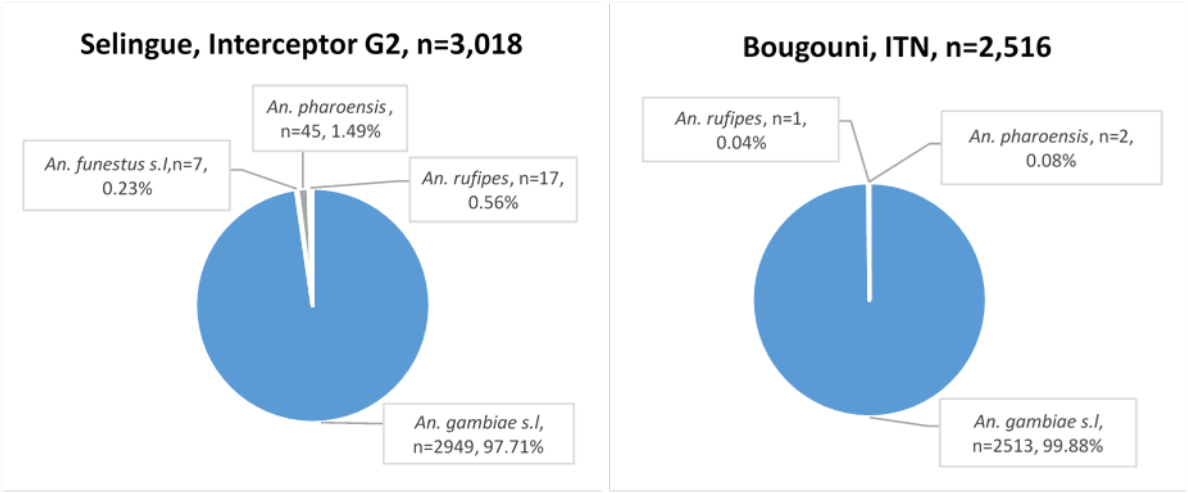


FIGURE 3: SPECIES COMPOSITION OF *ANOPHELES* COLLECTED BY HUMAN LANDING CATCH AND PYRETHRUM CATCHES IN THREE IRS SITES (BANDIAGARA, MOPTI AND DJENNÉ) AND THE UNSPRAYED CONTROL SITE (TOMINIAN) FROM JULY TO DECEMBER 2020 (TOTAL *ANOPHELES* COLLECTED = 12,278)



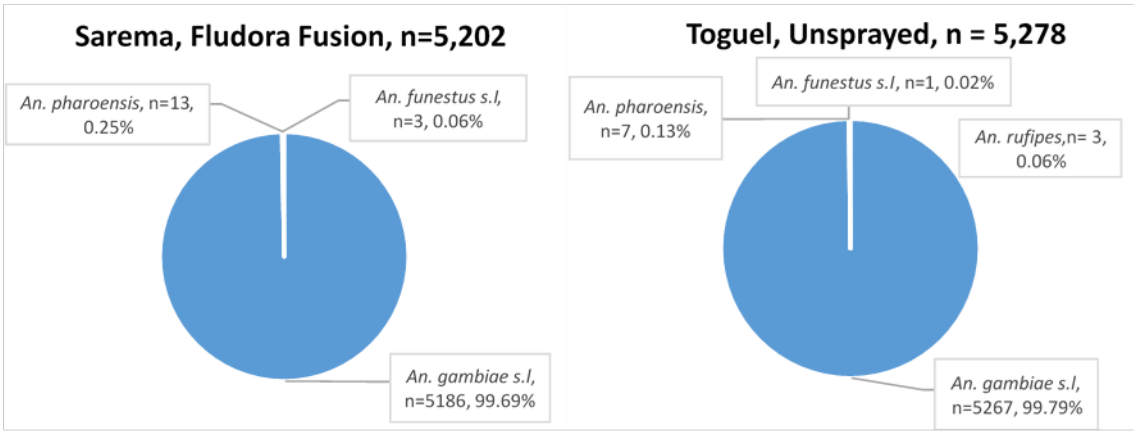
In Sikasso Region (Selingue and Bougouni sites) a total of 5,534 *Anopheles* were collected through PSC and HLC. Of the 5,534 *Anopheles* collected, 99% (5,462) were *An. gambiae* s.l., with the remainder being *An. pharoensis*, *An. rufipes* and *An. funestus* s.l. Unlike in Mopti Region where most were collected by PSC, approximately 79% (4,360) of all *Anopheles* were collected by HLC and only 21% (1,174) through PSC. It should be noted that Sikasso Region is more secure than Mopti, therefore night-time supervision of HLC was conducted by VectorLink staff, which was not possible in Mopti.

FIGURE 4: 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 AUGUST TO DECEMBER 2020 (TOTAL *ANOPHELES* COLLECTED = 5,534)



In the two sites in Mopti district where community-based surveillance was conducted using CDC light traps four *Anopheles* species were also collected, with 99.7% (10,453) *An. gambiae* s.l, and the rest *An. funestus* s.l., *An. pharoensis* and *An. rufipes* (Figure 5).

FIGURE 5: SPECIES COMPOSITION OF *ANOPHELES* COLLECTED BY CDC LIGHT TRAP CATCHES IN ONE SITE (SAREMA) SPRAYED WITH FLUDORA FUSION WP-SB AND ONE UNSPRAYED SITE (TOGUEL) IN MOPTI DISTRICT FROM AUGUST TO DECEMBER 2020 (TOTAL *ANOPHELES* COLLECTED = 10,480)

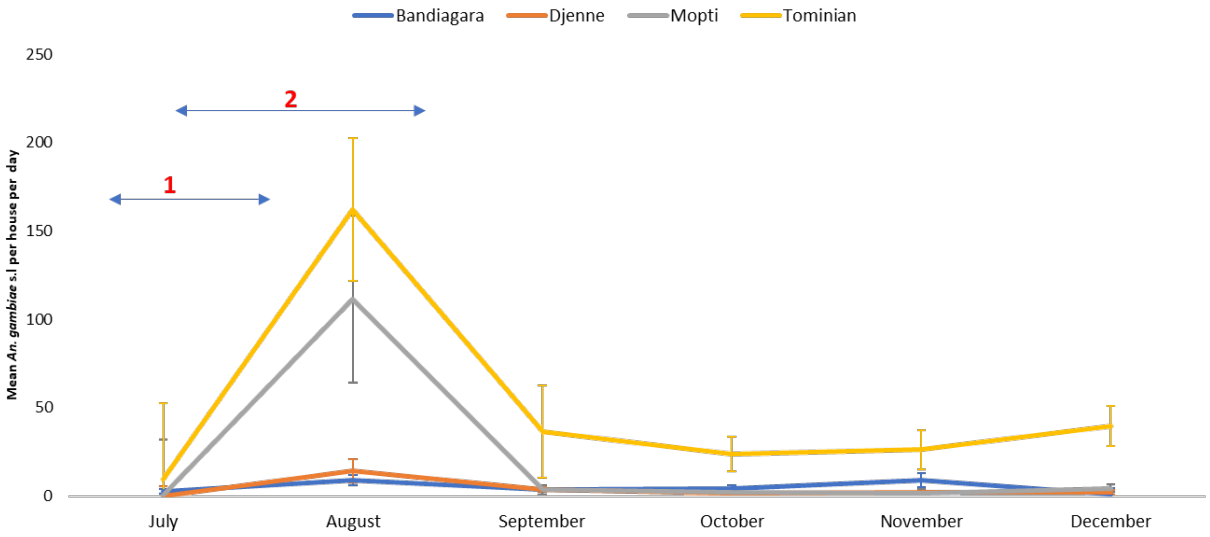


3.2 MALARIA VECTOR INDOOR RESTING DENSITY (BY PSC)

Figure 6 presents monthly PSC data for three sprayed sites in Mopti Region and one unsprayed site in neighboring Segou Region. The peak indoor resting density was observed in August in all districts, with the highest densities recorded in the unsprayed site of Tominian at 162 *An. gambiae* s.l. per house per day and in Mopti (Fludora Fusion) at 111 *An. gambiae* s.l. per house per day. Over the six-month monitoring period the

mean indoor resting density of *An. gambiae* s.l. was 4.1 per house per day in Djenné followed by 5.1 in Bandiagara, 20.7 in Mopti and highest in unsprayed Tominian with 49.8 *An. gambiae* s.l. per house per day.

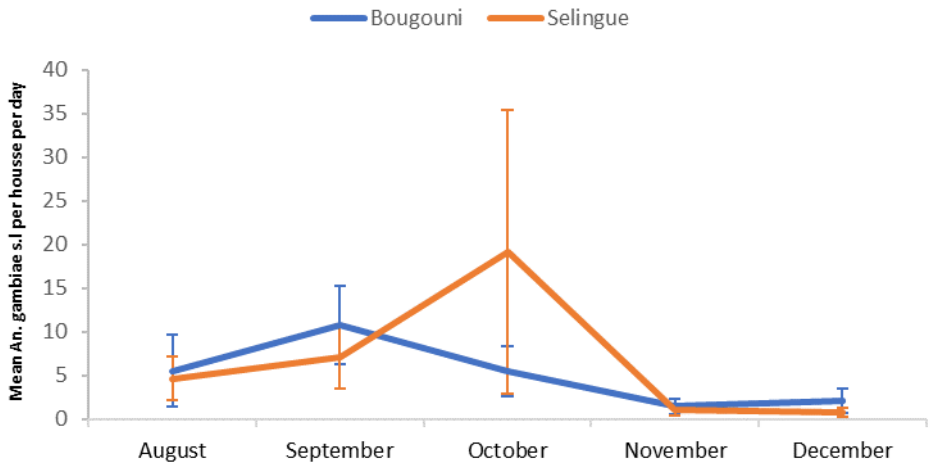
FIGURE 6: MEAN MONTHLY INDOOR RESTING DENSITY OF *AN. GAMBIAE* S.L. IN THREE IRS SITES (MOPTI, DJENNÉ AND BANDIAGARA) AND THE UNSPRAYED CONTROL SITE (TOMINIAN).



1: IRS campaign for Djenne and Bandiagara from June 15th to July 24th 2020.
2: IRS campaign for Mopti from June 29th to August 12th
Note: Error bars show 95% confidence intervals.

The peak indoor resting density in Bougouni (pyrethroid ITN) was observed in September with 10.8 *An. gambiae* s.l. per house per day and in Selingue (Interceptor G2) was October with 19.2 *An. gambiae* s.l. per house per day (Figure 7). The mean density over the five-month monitoring period was 5.1 *An. gambiae* s.l. per house per month in Bougouni and 6.7 *An. gambiae* s.l. per house per month for Selingue.

FIGURE 7: MONTHLY *ANOPHELES GAMBIAE* S.L INDOOR RESTING DENSITY IN BOUGOUNI (PYRETHROID ITN) AND SELINGUE (INTERCEPTOR G2).

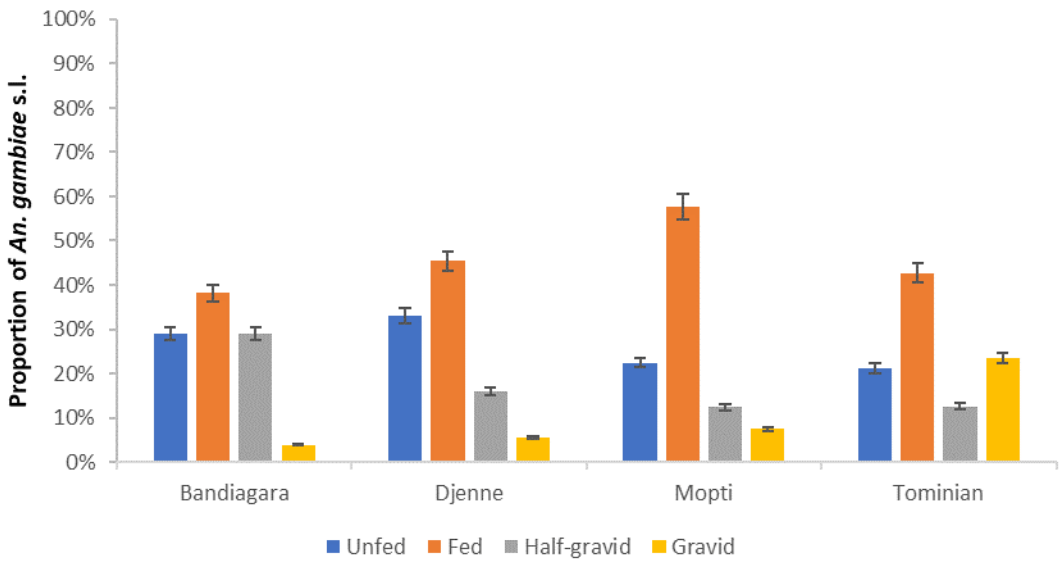


Note: Error bars show 95% confidence intervals.

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 three sprayed and one unsprayed site of is shown in Figure 8. In sprayed sites the percentage of gravid *An. gambiae* s.l. was 7% in Mopti followed by Djenne (6%), and Bandiagara (4%). The unsprayed site of Tominian had a relatively high percentage (25%) of gravid *An. gambiae* s.l. with 43% blood-fed. In the IRS sites most *An. gambiae* s.l. were unfed or blood-fed, with very few gravid. This was probably due to mosquitoes either being killed by insecticide or exiting earlier due to repellency before becoming gravid in sprayed sites.

FIGURE 8: ABDOMINAL STATUS OF *AN. GAMBIAE* S.L. COLLECTED BY PSC FROM JULY TO DECEMBER 2020 IN THREE IRS SITES (MOPTI, DJENNÉ AND BANDIAGARA) AND IN ONE UNSPRAYED SITE (TOMINIAN)

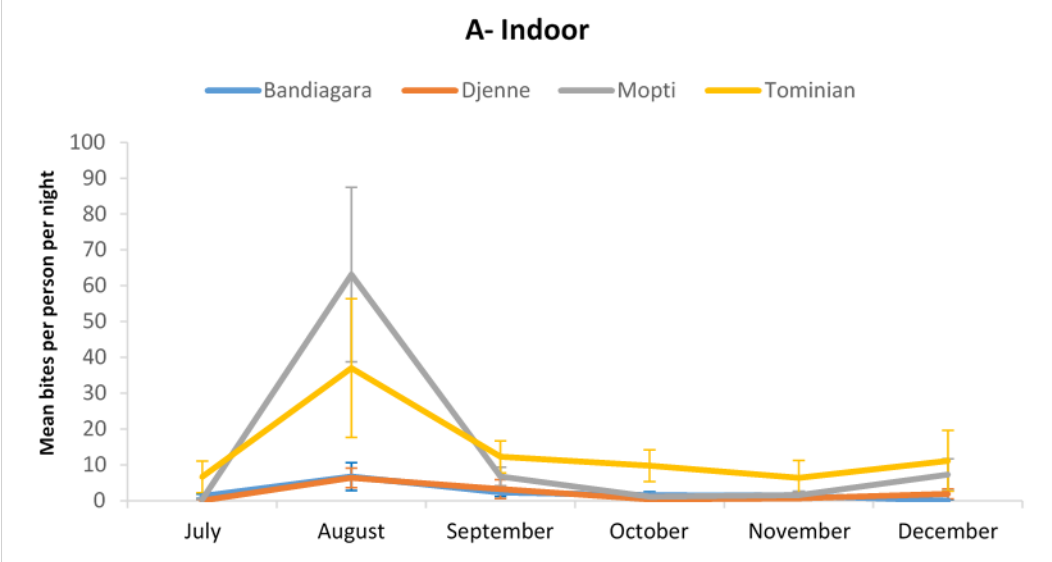


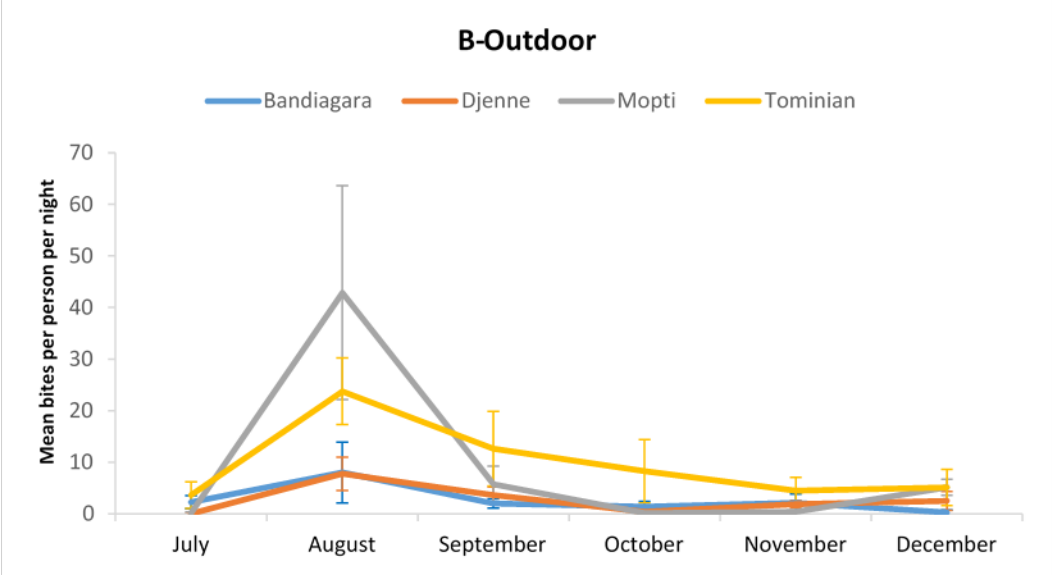
3.4 *AN. GAMBIAE* S.L. HUMAN BITING RATES

Figure 9 shows the mean monthly human biting rates for the three IRS sites and one control site. The peak biting rates were observed in August for all sites. The peak was highest in Mopti with 63.1 bites per person per night indoors and 42.9 b/p/n outdoors. Heavy rainfall occurred in Mopti district in August which may have led to the high densities in August and September. It should also be noted that spraying was delayed in Mopti by three weeks and was only completed in mid-August. The unsprayed control site of Tominian had a peak indoor biting rate of 37 b/p/n and 23.7 b/p/n outdoors in August. The peaks were lower in Djenné and Bandiagara.

Figure 10 shows the biting rates for two sites in Sikasso Region. The peak *An. gambiae* s.l biting rate was highest in Bougouni (pyrethroid ITNs) in September with 74.1 b/p/n indoor and 96.5 b/p/n outdoors. In Selingue (Interceptor G2 ITNs) the peak was recorded with 58.5 b/p/n indoors in August and with 52.9 b/p/n outdoors in September. The peak season was short, with *An. gambiae* s.l biting rates in Bougouni and Selingue below 1 b/p/n in November. The biting rates were generally higher than in Mopti Region. Mopti Region

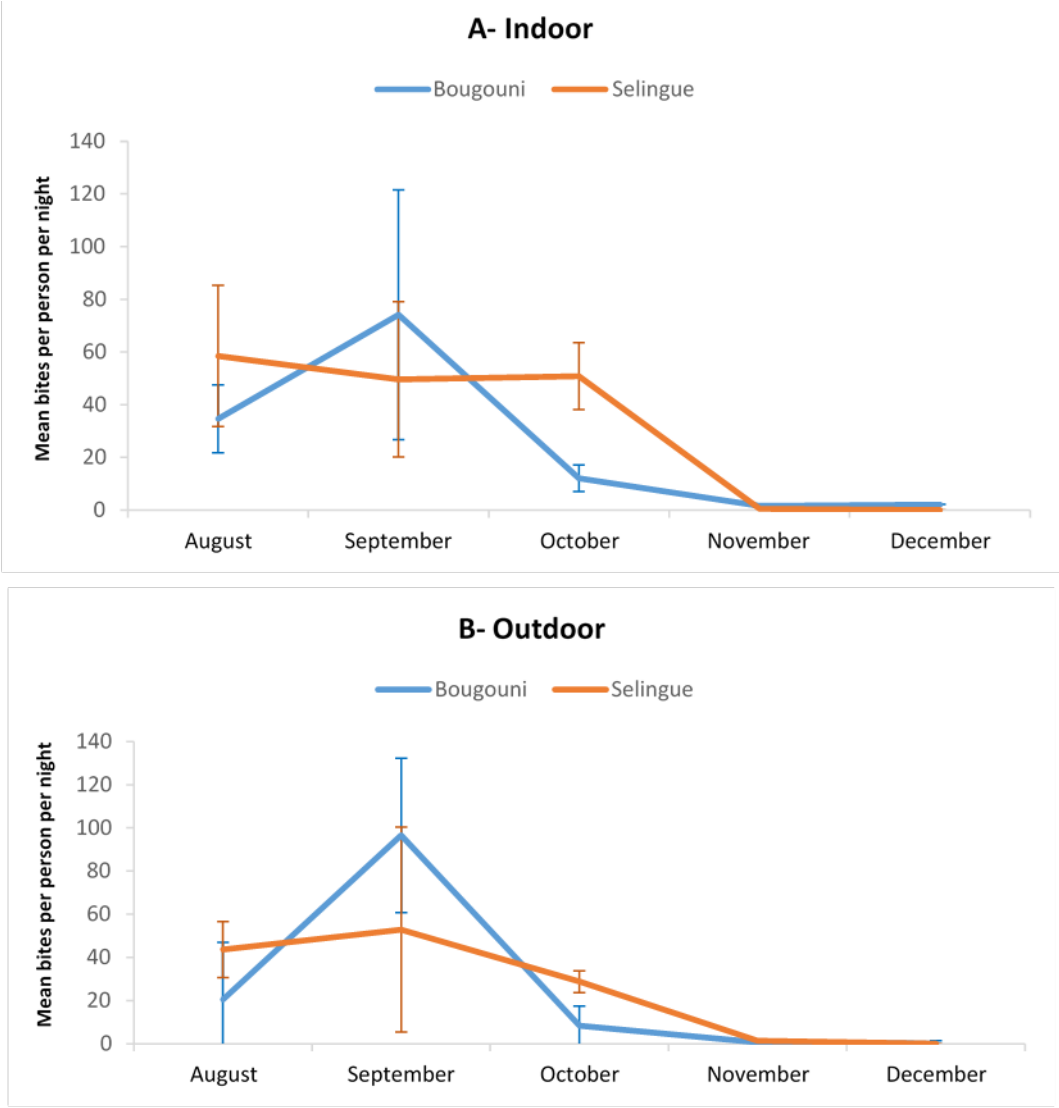
FIGURE 9: MONTHLY INDOOR (A) AND OUTDOOR (B) BITING RATES OF *AN. GAMBIAE* S.L. IN THREE IRS SITES (MOPTI, DJENNÉ AND BANDIAGARA) AND ONE UNSPRAYED SITE (TOMINIAN) FROM JULY TO DECEMBER 2020.





Note: Error bars show 95% confidence intervals.

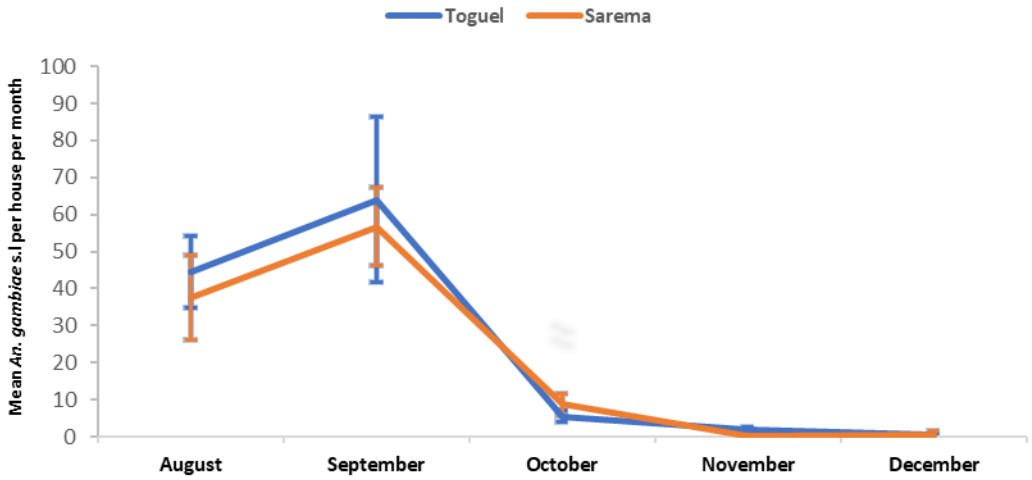
FIGURE 10: MONTHLY INDOOR (A) AND OUTDOOR (B) BITING RATES OF *AN. GAMBIAE* S.L. IN BOUGOUNI (PYRETHROID ITN) AND SELINGUE (INTERCEPTOR G2) FROM AUGUST TO DECEMBER 2020.



Note: Error bars show 95% confidence intervals.

Biting rates measured by proxy with indoor CDC-LT community-based surveillance for Sarema (Fludora Fusion) and Toguel (unsprayed) are presented in Figure 11. The peak biting rate was observed in September in both sites with 56.7 bites per night in Sarema and 63.9 in Toguel. Trends for sprayed Sarema and unsprayed Toguel were similar, with no clear impact of spraying on biting rates (although we have no baseline data before spraying). It should also be noted that IRS was delayed in Mopti district by three weeks and was not completed until mid-August. The data showed the peak season was short and by November biting rates in both sites were very low. Sarema was also the village in Mopti site used for HLC, therefore trends by HLC (Figure 9) and CDC-LT (Figure 11) can be compared. Both HLC and CDC-LT showed a biting peak in August, but only CDC-LT detected the continued biting peak through September, most likely due to the greater sampling effort (20 nights per month by CDC-LT compared with 2 nights per month for HLC).

FIGURE 11: MONTHLY *AN. GAMBIAE* S.L BITING RATE MEASURED BY CDC LIGHT TRAP IN TWO COMMUNITY-BASED SURVEILLANCE SITES IN MOPTI DISTRICT; TOGUEL (UNSPRAYED) AND SAREMA (FLUDORA FUSION WP-SB).

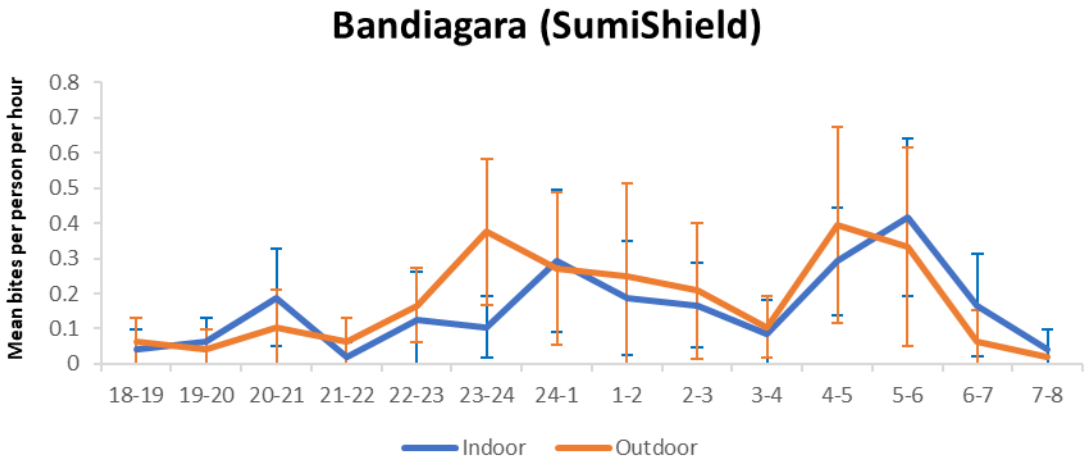


Note: Error bars show 95% confidence intervals.

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

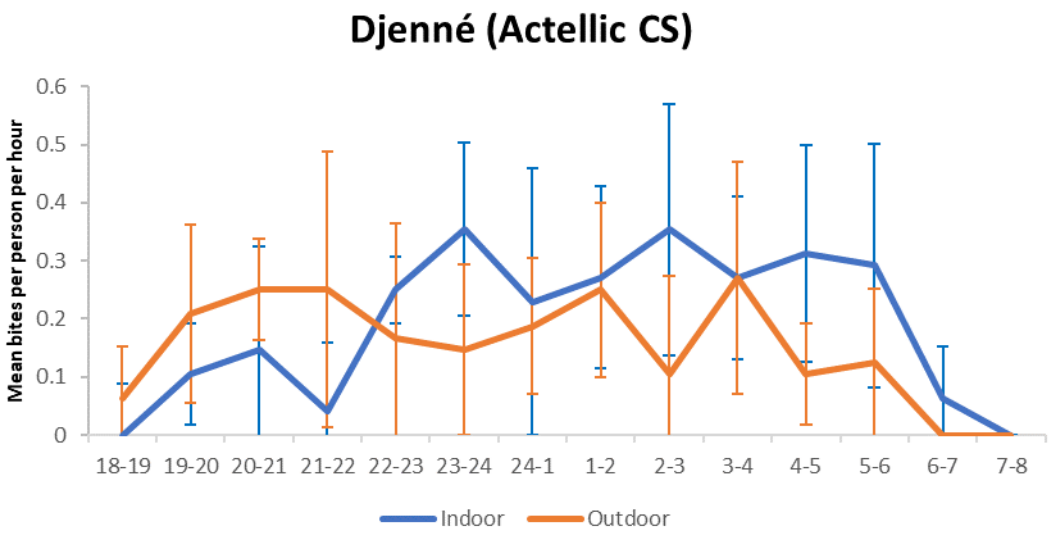
Figures 12 to 17 show the mean biting times of *An. gambiae* s.l. as recorded by HLC indoors and outdoors. The biting trends fluctuated quite a lot from hour to hour in all sites in Mopti Region but in general the highest biting rates were recorded late at night/early morning, particularly in Mopti where there was a major peak between 06:00-07:00 am and also in Tominian with a peak between 03:00 -06:00 am. In the two sites in Sikasso Region, the trends were clearer and there were two peaks in biting rates observed during the night. The first short peak was recorded at 20:00-21:00 pm indoors and outdoors with a prolonged second peak between 01:00-05:00 a.m.

FIGURE 12: MEAN *AN. GAMBIAE* S.L. HOURLY BITING RATES IN BANDIAGARA (SPRAYED WITH SUMISHIELD 50WG) FROM JULY TO DECEMBER 2020.



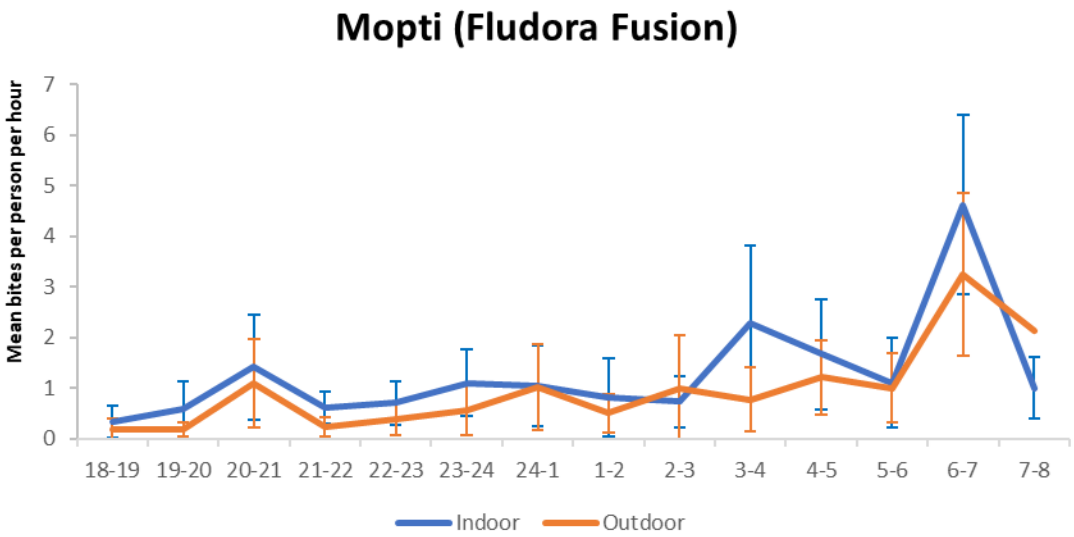
Note: Error bars show 95% confidence intervals.

FIGURE 13: MEAN *AN. GAMBIAE* S.L. HOURLY BITING RATES IN DJENNÉ (SPRAYED WITH ACTELLIC 300CS) FROM JULY TO DECEMBER 2020.



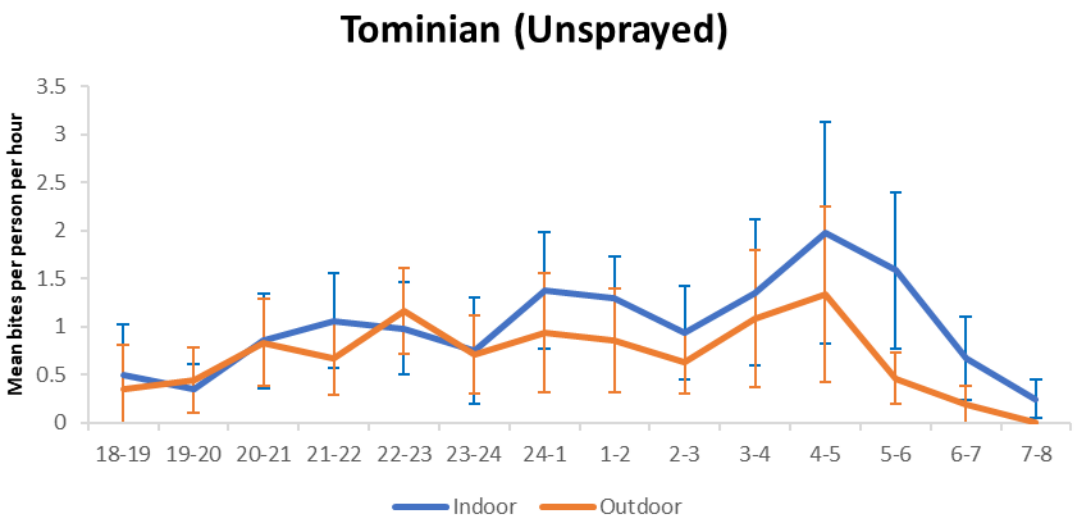
Note: Error bars show 95% confidence intervals.

FIGURE 14: MEAN *AN. GAMBIAE* S.L. HOURLY BITING RATES IN MOPTI (SPRAYED WITH FLUDORA FUSION WP-SB) FROM JULY TO DECEMBER 2020.



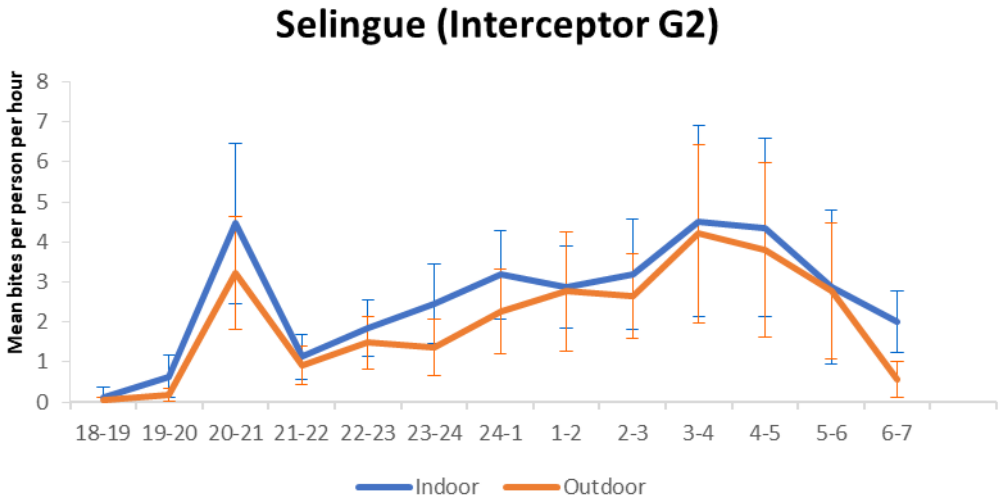
Note: Error bars show 95% confidence intervals.

FIGURE 15: MEAN *AN. GAMBIAE* S.L. HOURLY BITING RATES IN TOMINIAN (UNSPRAYED) FROM JULY TO DECEMBER 2020.



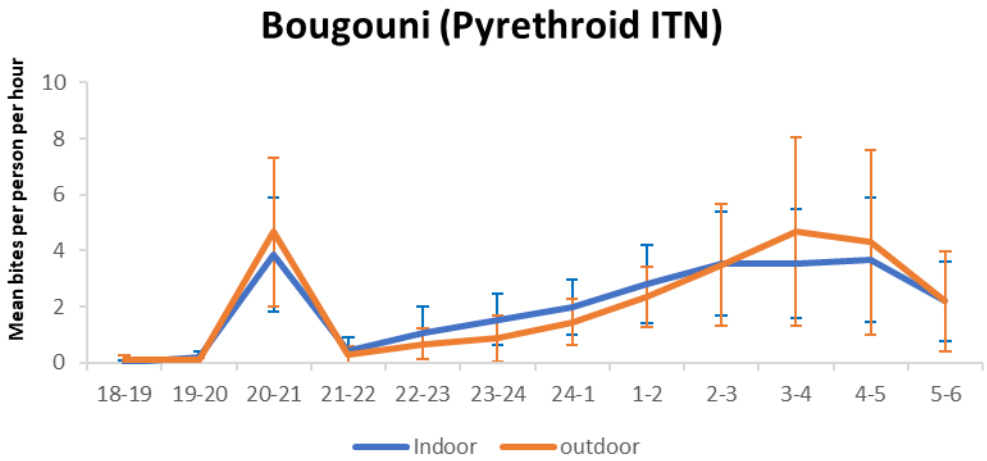
Note: Error bars show 95% confidence intervals.

FIGURE 16: MEAN *AN. GAMBIAE* S.L. HOURLY BITING RATES IN SELINGUE (INTERCEPTOR G2 ITN) FROM, AUGUST-DECEMBER 2020.



Note: Error bars show 95% confidence intervals.

FIGURE 17: MEAN *AN. GAMBIAE* S.L. HOURLY BITING RATES IN BOUGOUNI (PYRETHROID ITN) FROM AUGUST-DECEMBER 2020.



Note: Error bars show 95% confidence intervals.

3.6 PLASMODIUM SPOROZOITE RATE

Sporozoite ELISA results for *An. gambiae* s.l. collected by HLC are shown in Table 2. The mean sporozoite rate was highest in the unsprayed control site of Tominian with 3.73% (28/750). The lowest sporozoite rate was in Mopti with 0.28% (2/723), followed by Djenné with 2.21% (5/226) and Bandiagara with 2.24% (5/223). In Sikasso Region the sporozoite rate was highest in Bougouni (2.5% (15/600) which received pyrethroid only ITN in 2019 compared to 0.86% (5/581) in Selingue (Interceptor G2 nets) (Table 3). In both CDC LT sites (Toguel and Sarema), the sporozoite rate was the same at 050% (3/600). (Table 2).

TABLE 2: *PLASMODIUM FALCIPARUM* SPOROZOITE RATE IN *AN. GAMBIAE* S.L. PER SITE FROM JULY TO DECEMBER 2020 IN MOPTI REGION

Site, District (2020 Intervention)	Collection Method	Total *An. gambiae* s.l. Tested	% Mean Sporozoite Rate (positive/ tested)
Dandoly, Bandiagara (SumiShield 50WG)	HLC	223	2.24% (5/223)
Madiama, Djenné (Actellic 300 CS)	HLC	226	2.21% (5/226)
Sarema, Mopti (Fludora fusion SB-WP)	HLC	723	0.28% (2/723)
	CDC-LT (CBS)	600	0.50% (3/600)
Toguel, Mopti (Unsprayed control)	CDC-LT (CBS)	600	0.50% (3/600)
Ouena, Tominian (Unsprayed control)	HLC	750	3.73% (28/750)

TABLE 3: *PLASMODIUM FALCIPARUM* SPOROZOITE RATE IN *ANOPHELES GAMBIAE* S.L. PER SITE FROM AUGUST TO DECEMBER 2020 IN SIKASSO REGION

Site	Total *An. gambiae* s.l. Tested/ Total Collected by HLC	% Mean Sporozoite Rate (positive/tested)
Selingue (Interceptor G2)	581	0.86% (5/581)
Bougouni (Pyrethroid ITN)	600	2.50% (15/600)

3.7 ENTOMOLOGICAL INOCULATION RATE (EIR)

The indoor entomological inoculation rate was calculated by multiplying the mean human biting rate from HLC (or CDC LT) per night by the mean sporozoite rate and multiplying by the number of nights during the monitoring period (July to December = 180 days for HLC and August to December = 150 days for CDC LT). Results are presented in Table 4 for the IRS sites (Bandiagara, Djenné, Mopti) and unsprayed control sites (Tominian and Toguel) and in Table 5 for the two sites in Sikasso Region (Selingue and Bougouni). The highest EIRs were in the pyrethroid ITN site of Bougouni (119 infective bites per person in/p), the unsprayed sites of Tominian (114.1) and Toguel (Mopti) (14.7) and the sprayed site of Sarema (Mopti District) (14.4). In the IRS sites of Djenné and Bandiagara the EIR was relatively low at 13.1 and 10.8 infectious bites per person respectively. In Mopti site (HLC site) the mean indoor biting rate was high but the low sporozoite rate was the main driver behind the low EIR at 8.2 infectious bites per person.

In Sikasso Region, the EIR in Selingue (Interceptor G2 nets, 52.5 infectious bites per person) was half that of Bougouni (pyrethroid ITNs, 119.2 infectious bites per person) although as there was no baseline data collected we can't attribute the difference to the type of net. The difference was mainly driven by the lower sporozoite rate in Selingue than Bougouni, with biting rates remaining high.

TABLE 4: *AN. GAMBIAE* S.L. INDOOR ENTOMOLOGICAL INOCULATION RATE FOR 6 MONTHS (JULY TO DECEMBER 2020) IN FOUR IRS SITES AND TWO UNSPRAYED SITES IN MOPTI REGION

	Dandoly, Bandiagara (SumiShield)	Sarema, Mopti (HLC) (Fludora Fusion)	Sarema, Mopti (CDC LT) (Fludora Fusion)	Toguel, Mopti (CDC LT) (unsprayed control)	Madiama, Djenné (Actellic 300CS)	Ouena. Tominian (unsprayed control)
Mean indoor HBR per night	2.62	16.00	19.21	19.73	3.23	16.63
Sporozoite rate	2.24%	0.28%	0.50%	0.50%	2.21%	3.73%
EIR per night	0.059	0.045	0.096	0.098	0.071	0.620
EIR July to December 2020 (184 nights)	10.8	8.2	NA	NA	13.1	114.1
EIR August to December 2020 (150 nights)	NA	NA	14.4	14.7	NA	NA

NA = not applicable

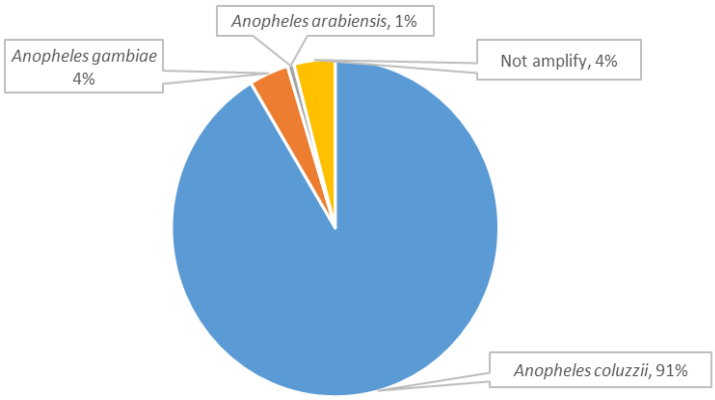
TABLE 5: *AN. GAMBIAE* S.L. INDOOR ENTOMOLOGICAL INOCULATION RATE (AUGUST TO DECEMBER 2020) FOR TWO SITES IN SIKASSO REGION

	Selingue (Interceptor G2)	Bougouni (Pyrethroid net)
Mean indoor HBR per night	39.88	31.16
Sporozoite rate	0.86%	2.50%
EIR per night	0.343	0.779
EIR August to December 2020 (153 nights)	52.5	119.2

3.8 MOLECULAR SPECIES IDENTIFICATION OF THE ANOPHELES GAMBIAE SPECIES COMPLEX

Out of a subsample of 638 *An. gambiae* s.l. from the longitudinal entomological monitoring in Mopti Region, 91.8% were identified by PCR as *An. coluzzii.* (n = 586), 3.9% as *An. gambiae* (n = 25), 0.7% as *An. arabiensis* (n = 1) and 4.1% did not amplify (n =26) (Figure 18).

FIGURE 18: PCR SPECIES COMPOSITION OF *AN. GAMBIAE* S.L. FROM 3 IRS SITES (DJENNÉ, MOPTI & BANDIAGARA) AND ONE CONTROL SITE (TOMINIAN)



Of the 150 *An. gambiae* s.l from Selingue and Bougouni tested by PCR, 91% in Selinguewere *An. coluzzii*, 3% *An. arabiensis* and 1% *An. gambiae*. In Bougouni, 81% were *An. gambiae*. 13% *An. coluzzii* and 1% *An. arabiensis*.

FIGURE 19: MOLECULAR SPECIES COMPOSITION OF *AN. GAMBIAE* S.L. FROM 2 SITES IN SIKASSO REGION (SELINGUE WITH INTERCEPTOR G2 AND BOUGOUNI WITH PYRETHROID ITNS) FROM AUGUST TO DECEMBER 2020

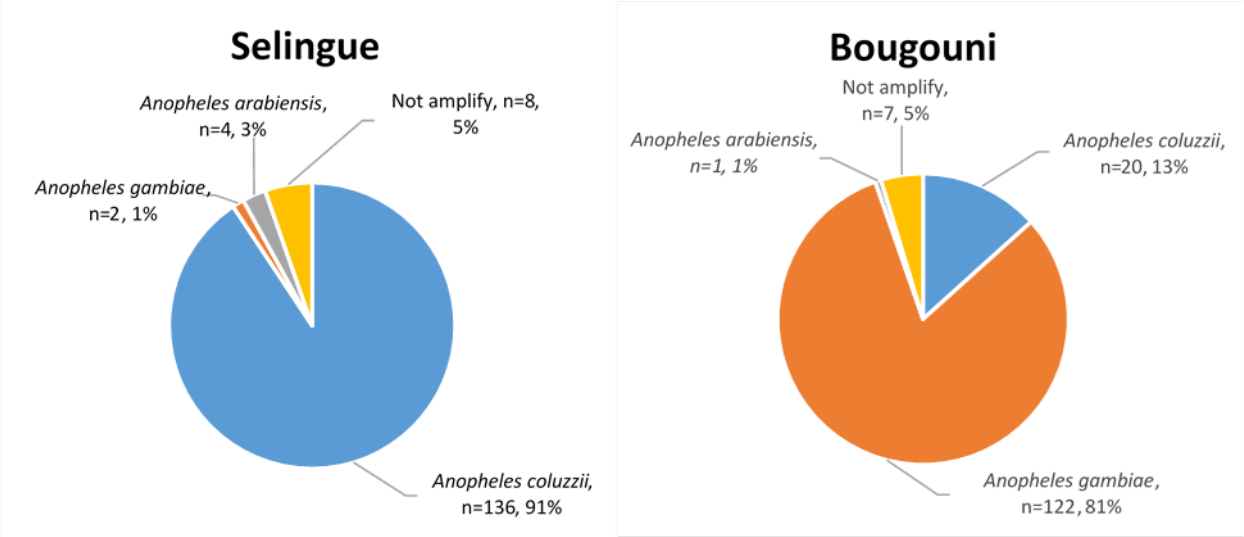
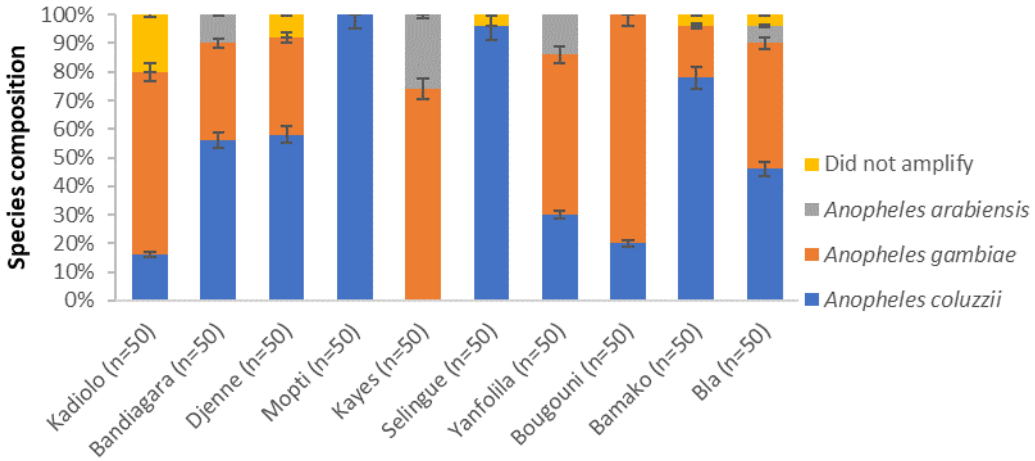


Figure 20 presents species composition in the ten sites where insecticide susceptibility tests were conducted. *Anopheles coluzzii* was the predominant species used in WHO susceptibility tests (collected as larvae) in 4 of 10 sites (Kadiolo, Kayes, Yanfolila, Bougouni) and *Anopheles gambiae* was predominant in 3 sites (Mopti, Selingue, Bamako), while there were similar proportions of *An. gambiae* and *An. coluzzii* in Bla, Bandiagara and Djenné. The number of samples that did not amplify was low (5%) overall in both sites (Figure 19).

FIGURE 20: MOLECULAR SPECIES COMPOSITION OF *AN. GAMBIAE* S.L. COLLECTED AS LARVAE FOR WHO SUSCEPTIBILITY TESTS, 2020



A total of 300 samples collected from August through December in Sarema and Toguel from the CBS sites were tested with *An. coluzzii* (298/300) the only species present.

3.9 FREQUENCY OF THE VGSC-L1014F, L1014S AND ACE-1 ALLELES IN *AN. GAMBIAE* S.L.

The frequency of the *Vgsc*-1014F mutation was high except in Kayes and Bandiagara with 0.29 and 0.36 respectively.. It was widespread in the rest of the sites at a frequency greater than 0.6 (Table 6) and found in both *An. gambiae* and *An. coluzzii*.

TABLE 6: FREQUENCY OF THE *VGSC* MUTATION 1014F (FORMERLY KDR-WEST) IN *AN. GAMBIAE* AND *AN. COLUZZII*

Site	Number Tested	RR (Homozygous resistant)	RS (Heterozygous resistant)	SS (Susceptible wild type)	Did Not Amplify	L1014F Frequency (of those that amplified)
Mopti	50	41	1	6	2	0.86
Bougouni	50	50	0	0	0	1.00
Kadiolo	50	40	5	4	1	0.86
Bamako	50	35	4	4	7	0.86
Djenné	50	23	9	13	5	0.61
Kayes	50	7	11	24	8	0.29
Bla	50	37	7	6	0	0.81
Badiangara	50	3	29	16	2	0.36
Selingue	50	41	6	0	3	0.93
Yanfolila	50	33	8	2	7	0.86

The *Vgsc*-L1014S mutation (formerly kdr-east), was not detected in 2019 but in 2020 was found in 5 out of 10 sites at low frequency (less than 0.1) (Table 7). The frequency of the *Ace 1R G119S* mutation was low <2% (Table 8).

TABLE 7: FREQUENCY OF THE *VGSC* MUTATION 1014S (FORMERLY KDR-EAST) IN *AN. GAMBIAE* AND *AN. COLUZZII*

Site	Number Tested	RR (Homozygous resistant)	RS (Heterozygous resistant)	SS (Susceptible wild type)	Did Not Amplify	L1014F Frequency (for those that amplified)
Mopti	50	0	0	49	1	0.00
Bougouni	50	0	0	45	5	0.00
Kadiolo	50	1	0	47	2	0.02
Bamako	50	0	0	43	7	0.00
Djenné	50	2	5	40	3	0.09
Badiangara	50	1	0	46	3	0.02
Kayes	50	2	2	46	0	0.06
Bla	50	0	3	47	0	0.03
Selingue	50	4	2	44	0	0.10
Yanfolila	50	0	0	50	0	0.00

TABLE 8: FREQUENCY OF *ACE 1R G119S* MUTATIONS IN *AN. GAMBIAE* AND *AN. COLUZZII*

Site	Number Tested	RR (Homozygous resistant)	RS (Heterozygous resistant)	SS (Susceptible wild type)	Did Not Amplify	Frequency 1014F (for those that amplified)
Mopti	50	0	1	43	6	0.01
Djenné	50	0	0	47	3	0.00
Badiangara	50	0	8	30	12	0.10
Kayes	50	0	0	43	7	0.00

3.10 Residual Duration of Insecticide Formulations

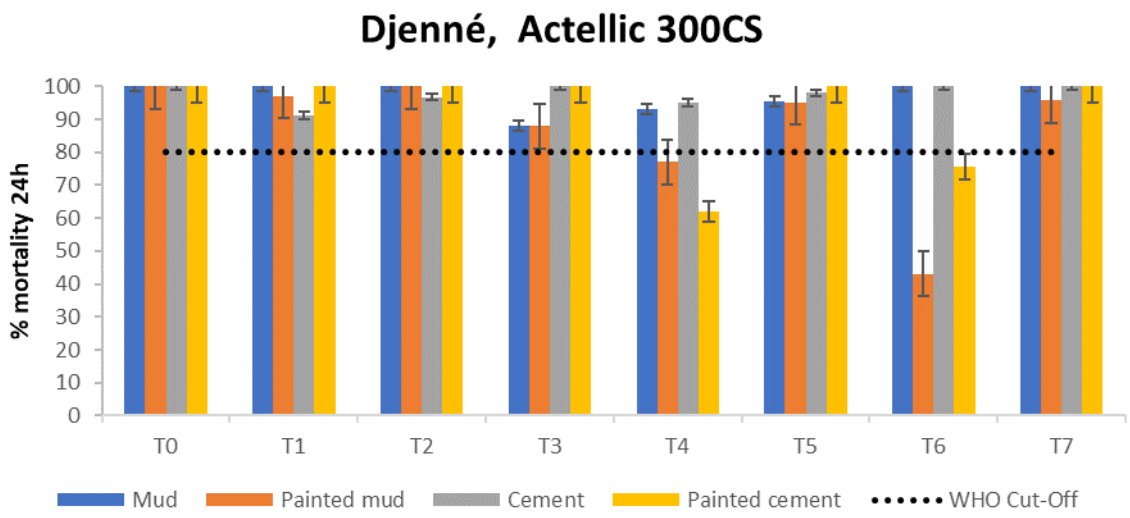
3.10.1 IRS Quality Assessment and Residual Efficacy Monitoring

WHO cone bioassays on sprayed walls at T0 (within five days of the start of spraying) produced 100% mortality for susceptible *An. coluzzii* 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 21-23).

Seven months after IRS in Djenné (sprayed with Actellic 300CS), the 24-hour mortality rate remained above 80% (WHO cut off), the latest available data at the time of this report. Some fluctuation was noted on painted mud and painted cement (Figure 21) with both surface types having two data points within the seven months below 80% mortality, but not in consecutive months. In Bandiagara (sprayed with SumiShield 50WG), the 72-hour mortality rate remained 100% seven months after spraying on all types of wall (Figure 22).

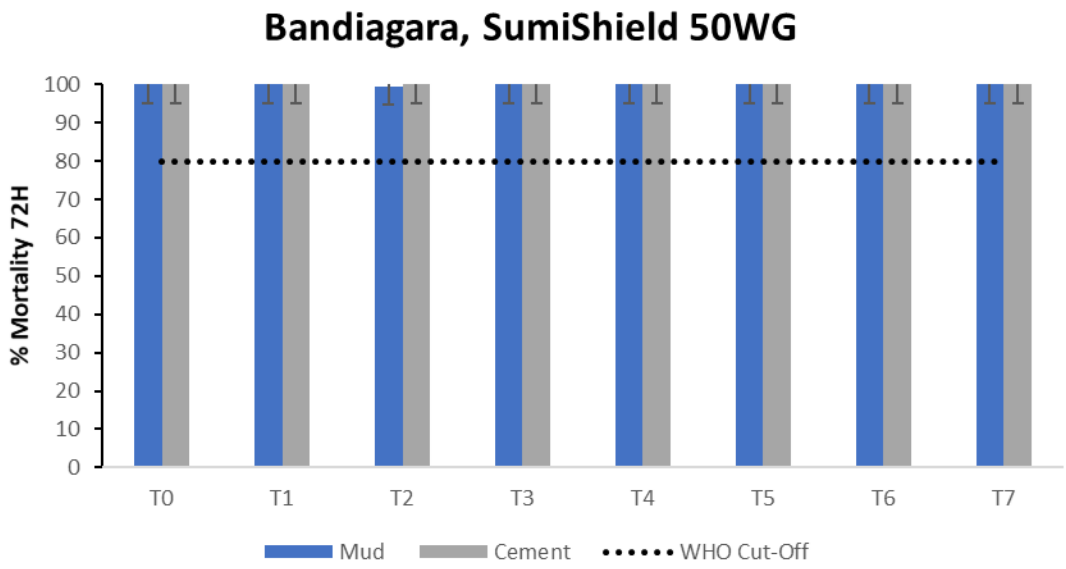
In Mopti site (sprayed with Fludora Fusion WP-SB), the 72-hour mortality rate with pyrethroid susceptible *An. coluzzii* remained above 80% after seven months on all the types of surfaces sprayed (Figure 23). In Mopti, wild pyrethroid resistant *An. gambiae* s.l. were tested from August to October (T2 to T4), for as long as wild larvae were available. The 72-hour mortality rate was above 95% on all the type of walls tested up to the fourth month after IRS, indicating that the clothianidin component of the formulation was effective (Figure 24).

Figure 21: Percentage Mortality (24h) from Cone Bioassay with Insectary Reared *An. coluzzii* (Ngousso strain) Tested on Walls Sprayed with Actellic 300CS in Djenné (Mopti Region)



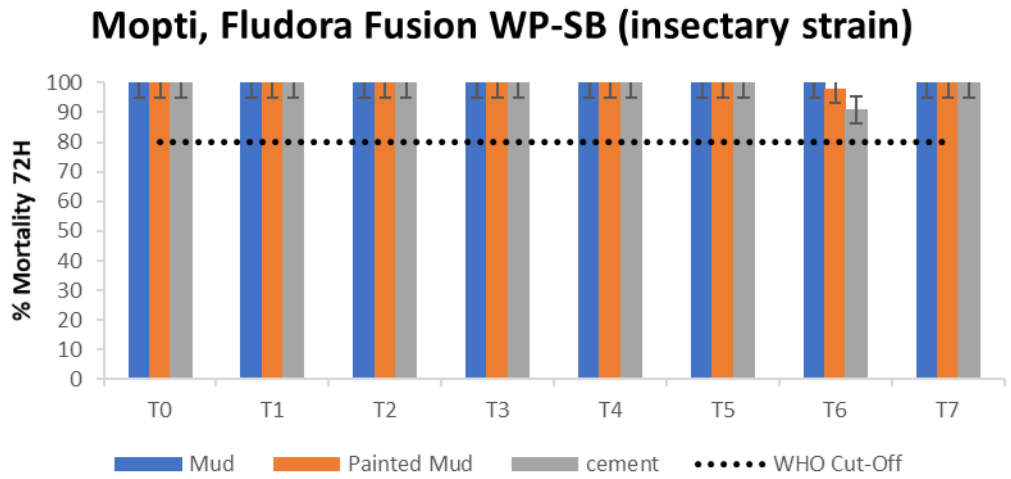
T = number of months after IRS campaign, e.g. T7 = seven months after IRS.

FIGURE 22: PERCENTAGE MORTALITY 72H AFTER CONE BIOASSAY WITH INSECTARY REARED *AN. COLUZZII* (NGOUSSO STRAIN) TESTED ON WALLS SPRAYED WITH SUMISHIELD 50WG IN BANDIAGARA



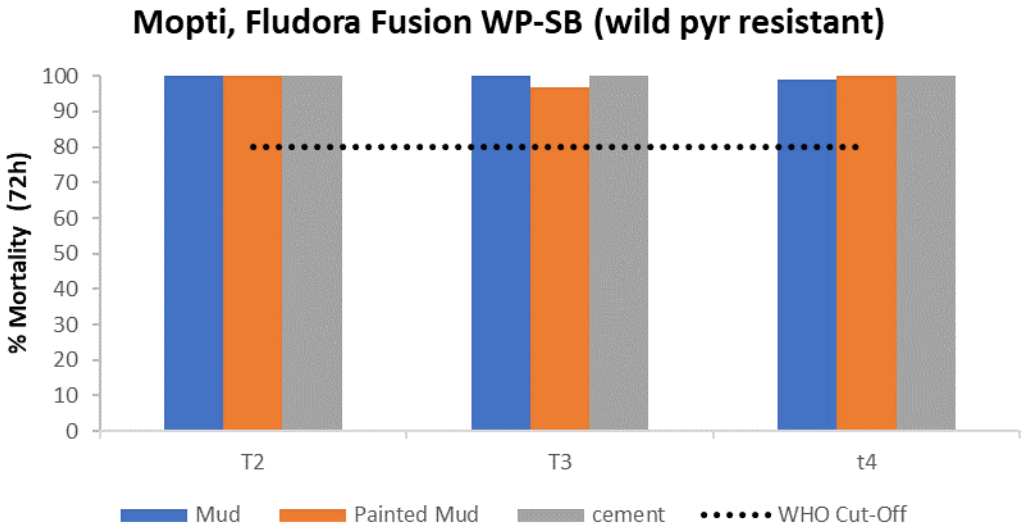
T shows the number of months after IRS campaign, e.g. T7 = seven months after IRS.

FIGURE 23: PERCENTAGE MORTALITY 72H AFTER CONE BIOASSAY WITH INSECTARY REARED *AN. COLUZZII* (NGOUSSO STRAIN) TESTED ON WALLS SPRAYED WITH FLUDORA FUSION WP-SB IN MOPTI DISTRICT



T = number of months after IRS campaign, e.g. T7 = seven months after IRS.

FIGURE 24: PERCENTAGE MORTALITY 72H AFTER CONE BIOASSAY WITH WILD *AN. GAMBIAE* S.L. (REARED FROM LARVAL COLLECTION IN MOPTI) TESTED ON WALLS SPRAYED WITH FLUDORA FUSION WP-SB IN MOPTI DISTRICT (MOPTI REGION) FROM AUGUST TO OCTOBER



T = number of months after IRS campaign, e.g. T7 = seven months after IRS.

3.10.2 FUMIGANT EFFECT

The mortality rate due to the airborne effect was very high within one week after IRS (T0) for Actellic 300CS, SumiShield 50WG and Fludora Fusion WP-SB. At T2, the mortality rate decreases in Djenné and Bandiagara but remained high in Mopti District. The fumigant effect appeared to last longer on structures with painted mud walls in Mopti, with greater than 50% mortality observed three months after spraying. In all other sites, the fumigant effect was short-lived and the mortality rate was less than 50% after 2-3 months (Figure 25-27).

FIGURE 25: FUMIGANT BIOASSAYS OF ACTELLIC 300CS IN DJENNÉ WITH INSECTARY REARED *AN. COLUZZII* (NGOUSSO STRAIN), JUNE–AUGUST 2020

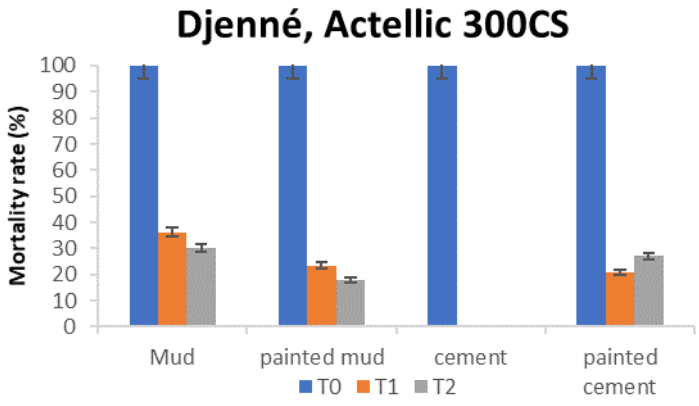


FIGURE 26: FUMIGANT BIOASSAYS WITH SUMISHIELD 50WG IN BANDIAGARA WITH INSECTARY REARED *AN. COLUZZII* (NGOUSSO STRAIN), JUNE–AUGUST 2020

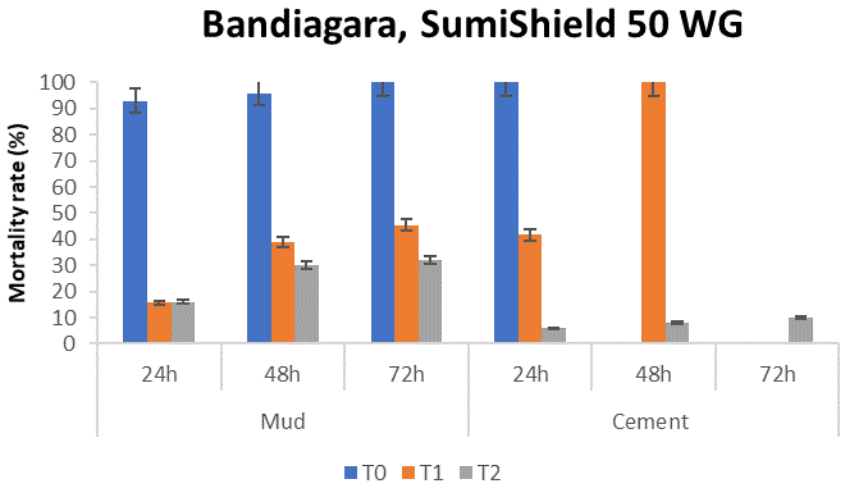
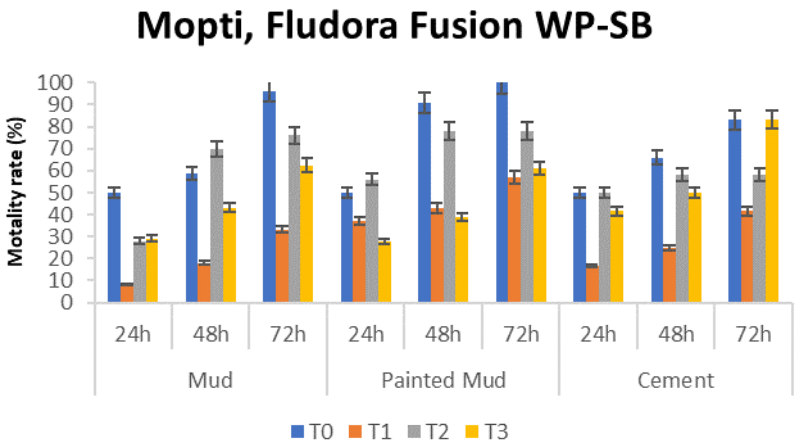


FIGURE 27: FUMIGANT BIOASSAYS WITH FLUDORA FUSION WP-SB IN MOPTI WITH INSECTARY REARED *AN. COLUZZII* (NGOUSSO STRAIN), JUNE- SEPTEMBER 2020

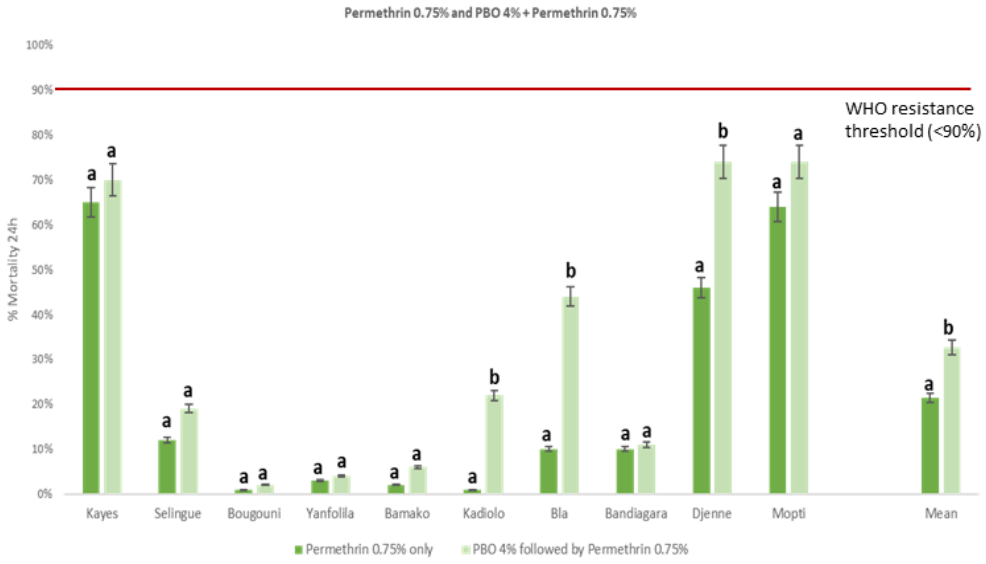


3.11 INSECTICIDE RESISTANCE MONITORING

Figures 28 to 30 show 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. According to WHO criteria, *An. gambiae* s.l. were resistant (mortality <90%) to the three pyrethroid insecticides (permethrin 0.75%, deltamethrin 0.05%, and alpha-cypermethrin 0.05%) tested in 2020 in all ten sites. Pyrethroid resistance intensity data was not collected because in previous years all sites showed high resistance intensity to permethrin, deltamethrin, and alpha-cypermethrin. Therefore, synergist tests and susceptibility tests for other IRS and ITN insecticide were prioritized in 2020.

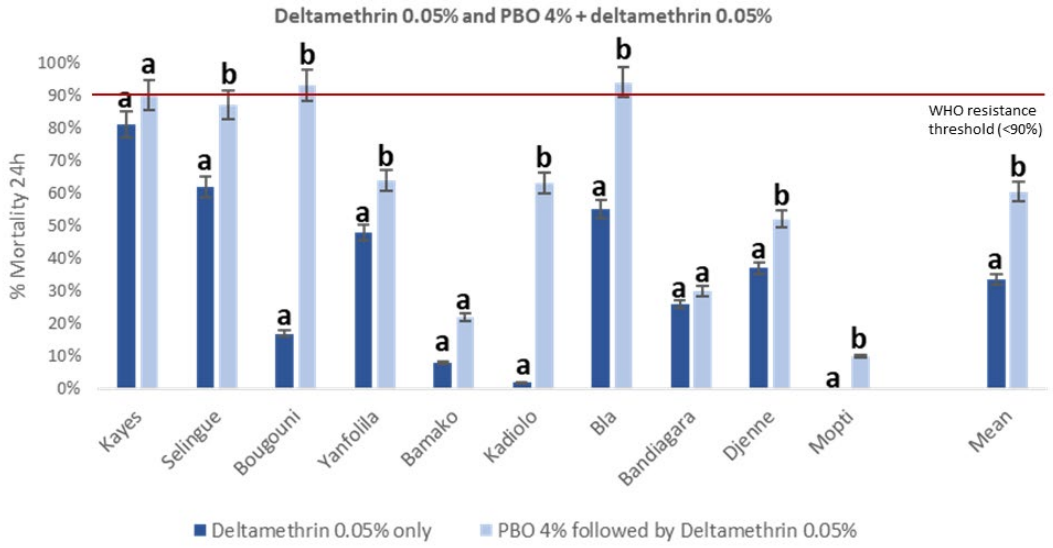
Pre-exposure to PBO generally resulted in increased mortality, but the degree varied by site and insecticide. There was a significant increase in mortality when testing with PBO + permethrin compared to permethrin only in 3 of 10 sites, 7 of 10 for PBO + deltamethrin and 6 of 10 for PBO + alpha-cypermethrin. These data suggest that PBO + permethrin nets would probably provide limited benefit over standard pyrethroid nets in most districts, but could be used in some districts such as Kayes, Djenne and Mopti where mortality exceeded 70%. These results showed the highest level of pyrethroid resistance was in Sikasso Region. In Bamako and Bandiagara mortality rates were extremely low for all three pyrethroids + PBO, therefore all types of PBO nets may not be suitable for distribution in these sites. In other sites, PBO + deltamethrin or alpha-cypermethrin, produced significant mortality although the mean mortality rate across all sites was only 45-60%. In Bougouni and Bla there were large significant (P<0.05) increases in mortality for PBO + deltamethrin compared to deltamethrin alone, meaning that PBO + deltamethrin nets may be suitable in these districts (Figure 29).

FIGURE 28: PERCENTAGE MORTALITY (24H) OF *AN. GAMBIAE* S.L. TESTED WITH PERMETHRIN 0.75% AND 4% PBO SYNERGIST + PERMETHRIN 0.75%



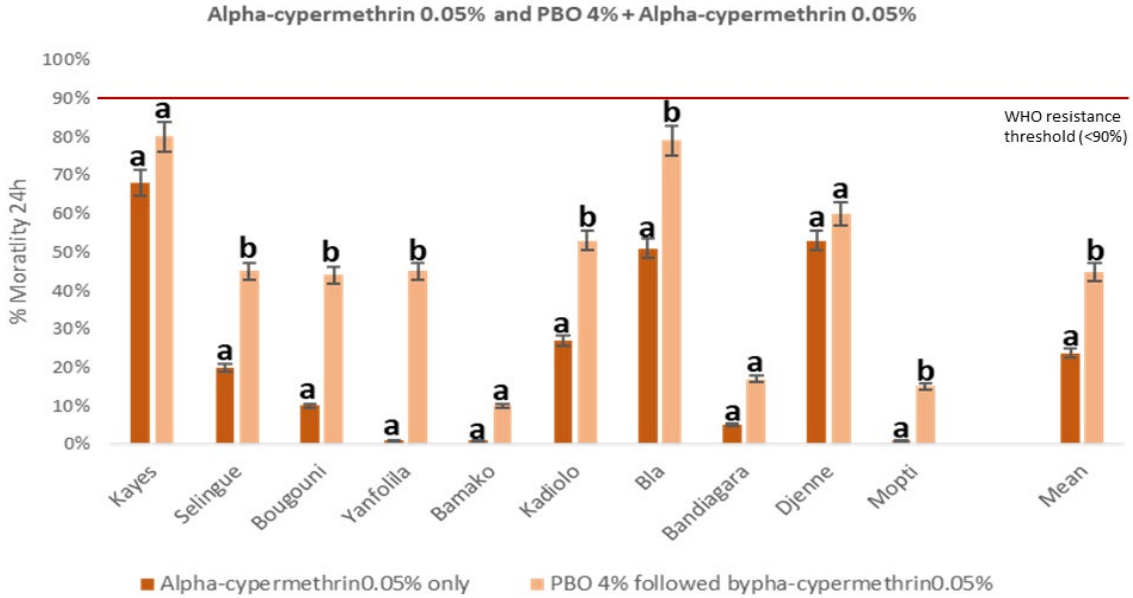
Superscript indicates whether % mortality for permethrin is significantly different to % mortality for permethrin + PBO.
a = no significant difference P>0.05; b = significant difference P<0.05

FIGURE 29: PERCENTAGE MORTALITY (24H) OF *AN. GAMBIAE* S.L. TESTED WITH DELTAMETHIN 0.05% AND 4% PBO SYNERGIST + DELTAMETHIN 0.05%



Superscript indicates whether % mortality for permethrin is significantly different to % mortality for permethrin + PBO.
a = no significant difference P>0.05; b = significant difference P<0.05

FIGURE 30: PERCENTAGE MORTALITY (24H) OF *AN. GAMBIAE* S.L. TESTED WITH ALPHA-CYPERMETHRIN 0.05% AND 4% PBO SYNERGIST + ALPHA-CYPERMETHRIN 0.05%



Superscript indicates whether % mortality for permethrin is significantly different to % mortality for permethrin + PBO.
A = no significant difference P>0.05; b = significant difference P<0.05

As WHO has not yet developed a guidance on susceptibility testing of chlorfenapyr, two doses (100µg/bottle and 200µg/bottle) were tested. 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 100µg dose, mortality was above 98% for wild *An. gambiae* s.l in 5 of 9 sites (Table

10). Full susceptibility was obtained in parallel tests for the susceptible *An. coluzzii* strain, confirming the validity of the tests.

Both wild *An. gambiae* s.l and the insectary strain of *An. coluzzii* showed susceptibility to chlorfenapyr at the dose of 200µg/bottle in 7 out of 9 sites performed after 72-hour holding period (Table 9). There were two sites with possible resistance (Bougouni 90% and Selingue 96%). Testing will be conducted again at these sites in 2021 to determine the validity of these results.

TABLE 9: PERCENTAGE MORTALITY (72H) OF WILD *AN. GAMBIAE* S.L. AND SUSCEPTIBLE INSECTARY *AN. COLUZZII* NGOUSSO STRAIN TESTED WITH CHLORFENAPYR (100 AND 200µG/BOTTLE) IN CDC BOTTLE BIOASSAYS

	100 µg/bottle		200µg/bottle	
	An. coluzzi susceptible strain	Wild *An. gambiae* s.l.	*An. coluzzi* susceptible strain	Wild *An. gambiae* s.l.
Bougouni	99%	78%	100%	90%
Bamako	99%	86%	100%	98%
Yanfolila	99%	99%	100%	100%
Selingue	99%	88%	100%	96%
Kadiolo	100%	100%	100%	100%
Mopti	100%	96%	100%	100%
Djenné	100%	100%	100%	100%
Bandiagara	100%	98%	100%	98%
Kayes	NP	NP	NP	NP
Bla	100%	100%	100%	100%

NP = not performed

In 2020, full vector susceptibility to 0.25% pirimiphos-methyl was found in 9 of 10 sentinel sites. It is the first time in Mali that resistance to this insecticide was observed in Kadiolo (86%). It is vital to repeat susceptibility testing for pirimiphos-methyl in this district in 2021 to confirm the finding.

Table 10 shows the mortality rates of wild *An. gambiae* s.l. populations following exposure to clothianidin 2% and parallel tests with the same papers using the susceptible insectary strain of *An. coluzzii*. *An. gambiae* s.l. were susceptible to clothianidin in all sentinel sites surveyed, including IRS sites of Mopti, Bandiagara and Djenné. One hundred percent mortality was recorded in 8 of 9 sites 3 days after exposure and 5 days after exposure in Djenné. Tests were not performed in Kayes due to high mortality of larvae collected in the field during transportation to Bamako. Tests were run on the same day, using the same treated papers, with the susceptible *An. coluzzii* strain to ensure there was no possibility of false resistance. One hundred percent mortality was recorded with the insectary strain in all sites 24h post exposure, confirming the quality of the treated papers.

TABLE 10: PERCENTAGE MORTALITY RATES OF *AN. GAMBIAE* S.L. (WILD) AND *AN. COLUZZII* (INSECTARY STRAIN), TESTED WITH CLOTHIANIDIN 2% (13.2 MG AI/PAPER)

Site	Mosquito source	% Mortality				
		24h	48h	72h	96h	120h
Bla	Insectary strain	100%				
	Wild	96%	99%	100%		
Kadiolo	Insectary strain	100%				
	Wild	99%	100%			
Bamako	Insectary strain	100%				
	Wild	97%	100%			
Bougouni	Insectary strain	100%				
	Wild	99%	100%			
Sélingué	Insectary strain	100%				
	Wild	96%	100%			
Yanfolila	Insectary strain	100%				
	Wild	98%	100%			
Kayes	Insectary strain	NP				
	Wild	NP				
Mopti	Insectary strain	100%				
	Wild	99%	100%			
Bandiagara	Insectary strain	100%				
	Wild	95%	100%			
Djenné	Insectary strain	100%				
	Wild	68%	85%	95%	98%	100%

NP = not performed

4. CONCLUSIONS

Evaluation of IRS efficacy through cone bioassays demonstrated that IRS was sufficiently dosed, with 100% mortality recorded on all types of wall and for all insecticide formulations sprayed. All three insecticide formulations provided control of an insectary strain of *An. coluzzii* for at least seven months (testing is ongoing until mortality falls below 80% for two consecutive months). According to these results the three insecticides (Actellic 300CS, SumiShield 50WG and Fludora Fusion WP-SB) provide control for the duration of the peak malaria transmission (for at least 4 months) and can continue to be used in the next IRS campaign in Mopti Region as part of a rotation strategy to mitigate the development of resistance.

The peak indoor resting densities and human biting rates occurred in August and September in all sites in Mopti and Segou regions, while in Sikasso Region the peak transmission season lasted from August to October, in agreement with the time when most mosquitos are captured. The pilot community-based surveillance was successful. The biting rates in Sikasso Region (Selingue and Bougouni) were generally higher than in Mopti Region (and there was a much higher ratio of *Anopheles* collected by HLC than PSC). These results could be due to a more secure situation in Sikasso which allowed for HLC supervision throughout the night by VectorLink staff, while in Mopti nighttime supervision was not possible between 23:00 and 06:00. Therefore, we think the biting rates in all sites in Mopti Region are likely to be an underestimate of actual biting risk. In 2021 we will not conduct HLC in Mopti Region but will replace it with CDC LT conducted by expanded community-based surveillance in 6 sites (3 sprayed, 3 unsprayed). Based on experience from 2020 we will adjust the sampling effort to be 12 nights for CDC LT per month and 6 for PSC (as 20 nights sampling per month resulted in a backlog of mosquitoes collected in the peak season).

The EIR was 119 infectious bites per person in Bougouni (pyrethroid ITN) and 52 infectious bites per person in Selingue (Interceptor G2 net). This is an encouraging sign but as there was no baseline data collected in 2019 we can't attribute the difference to the type of net distributed. The difference was mainly driven by the lower sporozoite rate in Selingue than Bougouni, with biting rates remaining high.

The EIR was higher in the unsprayed sites of Tominian (114 infectious bites per person) compared to neighboring Djenné (13 infectious bites per person). One of the reasons to this difference could be the impact of IRS. In Mopti District, the EIR was 14.7 infectious bites per person in Toguel (unsprayed) and 14.4 infectious bites per person in Sarema (sprayed with Fludora Fusion WP-SB) as measured by community-based surveillance.

According to WHO criteria, *An. gambiae* s.l. were resistant to the three pyrethroid insecticides (permethrin 0.75%, deltamethrin 0.05%, and alpha-cypermethrin 0.05%) tested in 2020 in all ten sites. Our results indicate that, PBO + permethrin nets can not be use nationwide, but may provide greater efficacy than standard pyrethroid ITNs in some districts such as Kayes, Djenne and Mopti. Wild *An. gambiae* s.l were susceptible to chlorfenapyr at the dose of 200µg/bottle in 7 out of 9 sites tested. Given the limited increase in mortality with PBO and susceptibility to chlorfenapyr in most sites, Interceptor G2 nets should be prioritized for future ITN campaigns in Mali. Clothianidin and pirimiphos-methyl formulations should continue to be used in rotation for insecticide resistance management in Mali based on their long residual action (at least 7 months) and continued susceptibility in malaria vectors.