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# PMI VECTORLINK MALI

## ANNUAL ENTOMOLOGICAL MONITORING REPORT

## JANUARY - DECEMBER 2019

**Recommended Citation:** The PMI VectorLink Project Mali, Annual Entomological Monitoring Report. January - December 2019. Rockville, MD. The PMI VectorLink Project, Abt Associates Inc.

**Contract:** AID-OAA-I-17-00008

**Task Order:** AID-OAA-TO-17-00027

**Submitted to:** United States Agency for International Development/PMI

**Submitted on:** 31 March 2020

**Approved on:**


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# PMI VECTORLINK MALI

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# 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** | U.S. President's Malaria Initiative |
| **PSC** | Pyrethrum spray catch |
| **WG** | Wettable granules |

# EXECUTIVE SUMMARY

The 2019 Mali indoor residual spraying (IRS) campaign was implemented from July 1st to August 4th, 2019, in 3 selected Health Districts in the Mali region. It covered a total of 148,198 structures (332,991 rooms) out of 153,191 structures (362,694 rooms) found by project spray operators in the targeted areas, accounting for a coverage rate of 96.7%. Actellic 300CS (capsule suspension) was sprayed in Bandiagara and Mopti districts for the third consecutive year and SumiShield 50WG was sprayed for the second year in Djenné district.

To monitor the impact of spraying, monthly cone bioassay was conducted on sprayed walls with an insectary reared susceptible strain of *An. coluzzii* Ngousso and entomological surveillance of wild mosquitoes was conducted in three sprayed sites (Mopti, Bandiagara, Djenné) using pyrethrum spray catch (PSC) and human landing catch (HLC). An unsprayed control site, Tominian in neighboring Segou Region, was monitored to allow for comparison of entomological indicators with sprayed sites. Insecticide susceptibility tests were conducted in 13 sites, to gather insecticide resistance information of local mosquito populations to inform the choice of insecticide for future IRS and ITN campaigns. Susceptibility tests using wild *Anopheles gambiae* s.l. were conducted with pyrethroid insecticides according to World Health Organization (WHO) protocols, with and without pre-exposure to the synergist piperonyl butoxide (PBO). WHO tube tests were also conducted to determine susceptibility to pirimiphos-methyl and clothianidin, both of which were used for IRS. CDC bottle bioassays were conducted to determine susceptibility status to chlorfenapyr.

IRS with Actellic 300CS in Bandiagara and Mopti had good residual efficacy, providing over 80% mortality (the WHO cut-off) for four months in Mopti and up to seven months in Bandiagara. Cone bioassays demonstrated that SumiShield 50WG provided a minimum of seven months residual control. As the main malaria transmission season is short in Mopti Region (lasting approximately 4 months), either Actellic 300CS or SumiShield 50WG can be used as part of a rotation strategy.

*An. gambiae* s.l. was the most frequently sampled species which accounted for 98.5% of *Anopheles* collected (10,324/10,481). The peak indoor resting densities were observed in September in all sites. The control site of Tominian had an extremely high density of 150 *An. gambiae* s.l. per house per day, while Djenné had the highest density among the sprayed sites (92 *An. gambiae* s.l. per house per day), only 2 months after IRS. Densities were lower (16 *An. gambiae* s.l. per house per day) in Mopti and 5 in Bandiagara at the peaks in September 2 months post IRS. The indoor resting density then decreased progressively between October and December in all sites as the dry season began, but high in the control site of Tominian than in sprayed sites. Indoor resting densities were higher in the site sprayed with SumiShield 50WG compared to those sprayed with Actellic 300CS. This may be due to the slower-acting nature of SumiShield 50WG, meaning that mosquitoes may survive long-enough to be captured by pyrethrum spray catch in the morning.

By the end of the dry season (May to June) the human biting rate was zero in all IRS sites. The average biting rate per person per night (b/p/n) was low during the rainy season in IRS sites sprayed with Actellic 300CS. Overall, the human biting rate was relatively low in all three IRS districts, regardless of the insecticide sprayed and was high in the unsprayed site of Tominian during the rainy season (July-October). In Tominian (unsprayed control site) the peak biting rate was observed in August and September and was significantly higher than all sprayed sites, reaching 31 b/p/n indoors in September and 19 b/p/n outdoors in August. Human biting rates were generally far lower than the mean indoor resting densities from PSC at all sites and biting rates may be underestimated. This may be related to not being able to provide night time supervision of HLCs due to insecurity.

The overall sporozoite rate  was loweer in Mopti (0.3%) and Bandiagara (0.1%) sprayed with Actellic 300CS compared with 1.1% in Djenné (sprayed with SumiShield 50WG) and 2.4% in Tominian (unsprayed).  . In all IRS sites, the EIR was relatively low  less than 5 infectious bites per person between May and December compared to the unsprayed site with an estimated EIR of 40-65 infectious bites per person between May and December. Despite limitations including only one unsprayed site and no baseline data for the IRS sites, the data suggest that IRS is having a positive effect in Mopti Region in terms of malaria vector abundance, biting and infectivity rates.

Mosquitoes tested were fully susceptibile to pirimiphos-methyl and clothianidin in all 13 sentinel sites including the IRS sites of Mopti, Bandiagara and Djenné. Therefore, based on malaria vector populations being susceptible, both Actellic 300CS (pirimiphos-methyl) and insecticide formulations containing clothianidin SumiShield 50 WG and Fludora Fusion WP-SB) can be considered for IRS as part of a rotation strategy for insecticide resistance management.  *An. gambiae* s.l. was resistant to the three pyrethroid insecticides commonly used on ITNs (permethrin, deltamethrin and alpha-cypermethrin). PBO synergist tests implicated mixed function oxidases (MFOs) in pyrethroid resistance. Significantly increased in *An. gambiae* s.l. mortality rates were obtained in all sites with these three pyrethroids after pre-exposure to PBO, although mortality was generally still below 90%. Wild *An. gambiae* s.l. from 10 of 12 sites showed susceptibility to chlorfenapyr in bottle bioassays at a dose of 200µg/bottle. Due to the widespread presence of pyrethroid resistance and limited impact of PBO synergists, next generation ITNs such as Interceptor G2 (containing chlorfenapyr + pyrethroid) should be prioritized for future ITN distribution campaigns. The 'New Nets Project' with 2 million Interceptor G2 nets should provide additional evidence on the cost-effectiveness of the use of the dual active ingredient nets in Mali.

# 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. PMI supports the National Malaria Control Program's (NMCP) strategy to reduce malaria transmission through targeted IRS in select high-risk areas. Starting in 2008, PMI supported three IRS campaigns in the districts of Bla and Koulikoro, adding a third district (Baraoueli) in 2011. In 2015, PMI reduced the IRS sites to two districts because of the added cost of spraying a new insecticide formulation, Actellic 300CS, to help mitigate pyrethroid resistance in *An gambiae* s.l.. In 2016, Mali benefited from the UNITAID-funded Next Generation Indoor Residual Spraying (NGenIRS) project, which included a short-term co-payment on long-lasting IRS insecticides. This allowed IRS expansion coverage back to three districts (Koulikoro, Baroueli, and Fana). Since 2017, PMI shifted IRS operations from these three districts in the south of Mali to Mopti Region in central Mali. This was based on the 2015 epidemiological data showing a 60 percent malaria prevalence rate in this region compared to 30 percent national average. In addition to its higher malaria prevalence, Mopti Region did not benefit from the same level of malaria control resources as other areas.

The primary objective of the VectorLink Mali project in 2019 was to reduce malaria-associated morbidity and mortality in three districts of Mopti Region (Mopti, Bandiagara, and Djenné). As recommended by the Mali NMCP vector control policy, an insecticide rotation strategy was established for resistance management. As such, Actellic® 300CS was used in Bandiagara and Mopti, and SumiShield® 50WG in Djenné. Over the period of July 1 to August 4, 2019, the project sprayed a total of 148,198 structures (332,991 rooms) out of 153,191 (362,694 rooms) found by project spray operators in the targeted areas, accounting for a coverage rate of 96.7%, which is highly remarkable despite highly unstable security conditions.

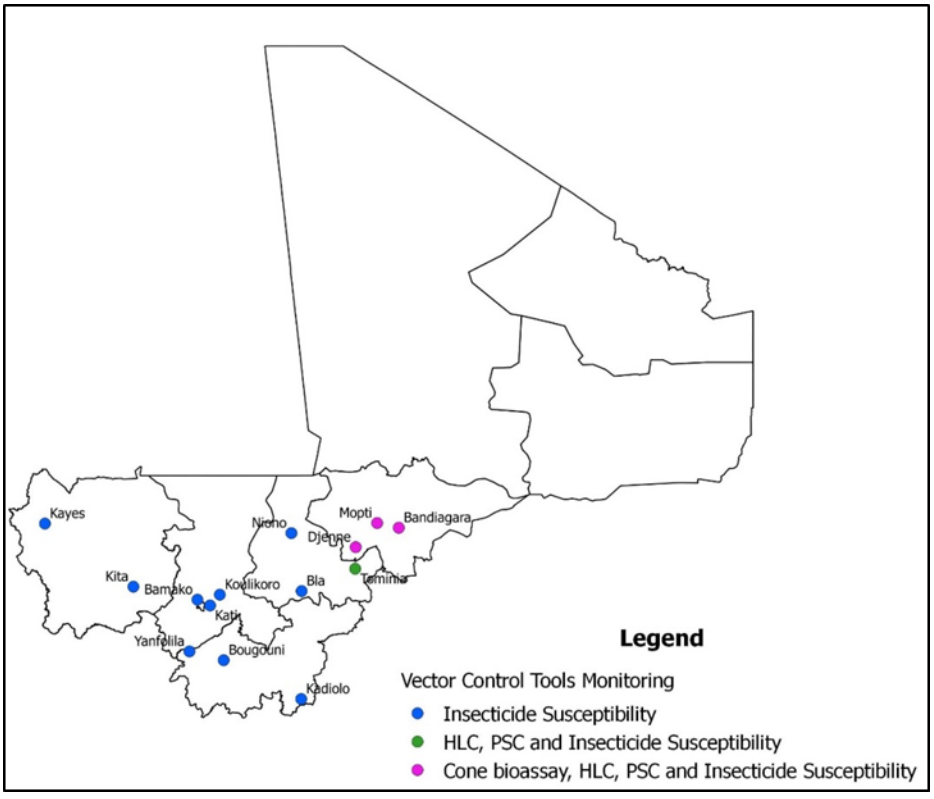
Monthly entomological monitoring using human landing catch (HLC) and pyrethrum spray catch (PSC) was conducted from May to December 2019 in three IRS sites in Mopti Region and one unsprayed control site in Tominian (Segou Region, around 50km from the Mopti Region). Insecticide susceptibility monitoring was conducted in 13 sites nationwide (southern and central Mali) to determine the susceptibility status of *An. gambiae* s.l. to pyrethroid, organophosphate, neonicotinoid and pyrrole insecticides and to conduct PBO synergist assays. The location of the sites is shown in Figure 1. Due to insecurity in parts of Mopti Region, the site of Bankass, which has previously been monitored, was not monitored for susceptibility or longitudinal surveillance in 2019. The results of insecticides resistance monitoring will guide the NMCP in the choice of insecticides for IRS and ITNs.

# 2. METHODOLOGY

## 2.1 STUDY AREA

Longitudinal vector surveillance and insecticide resistance monitoring was conducted in three IRS sites (Mopti, Bandiagara and Djenné) and one unsprayed control site (Tominian). Insecticide resistance data (including PBO synergist data) was also collected from a total of 13 sites. The locations consist of the 3 IRS sites (Mopti, Bandiagara, Djenné), 1 unsprayed control site (Tominian), 2 former IRS districts (Koulikoro and Bla) and 8 other sites chosen in collaboration with the NMCP, The latter represent the most populated regions of the country: Kayes, Kita, Kati, Bamako, Yanfolila, Kadiolo, Bougouni and Niono (Figure 1).

**Figure 1. Map of Mali showing regional boundaries and 2019 entomological surveillance sites.**




## 2.2 LONGITUDINAL MONITORING

Entomological monitoring was conducted monthly from May to December 2019 in three IRS sites (Mopti, Bandiagara and Djenné) and one control site (Tominian) (Table 1). Monitoring was also scheduled for two unsprayed control sites in Mopti (Diambacoura village) and Bandiagara (Tabitongo village) but the later was

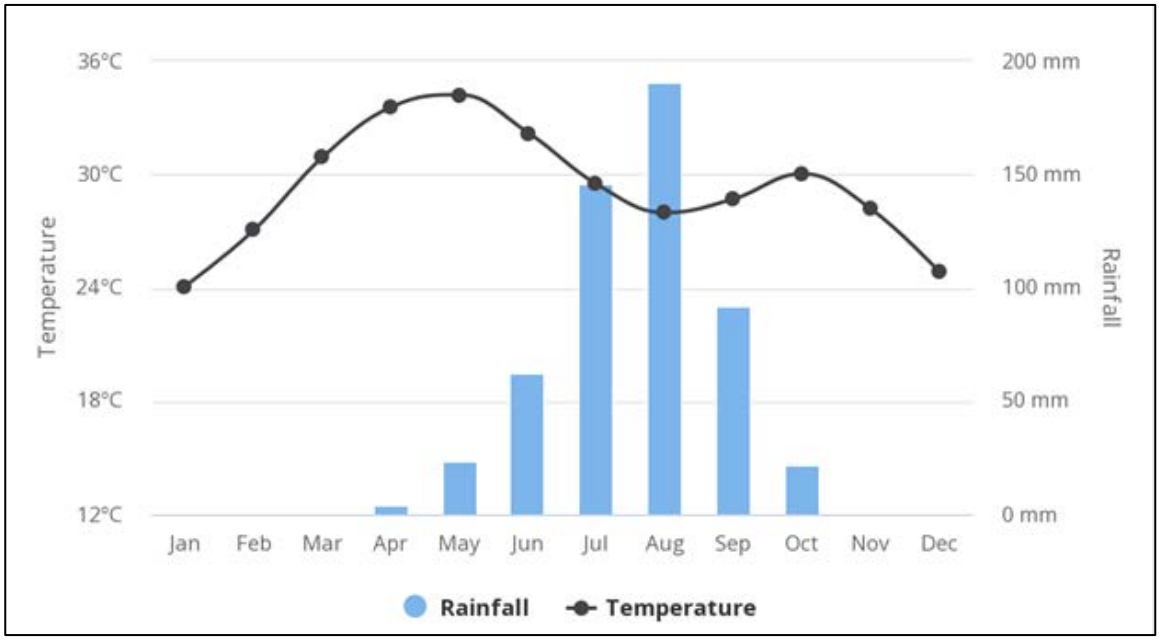
(HLC) and pyrethrum spray catch (PSC). Mean climate data for Mopti District in Figure 2 shows the rainy season is short, lasting 3-4 months from June to September, with mean lower and upper temperatures of 24 and 34°C respectively.

**Table 1. Monthly entomological surveillance sites (May to December, 2019).**

| Region | District | Health Area | Site (village) | Spray Status | Geographic Zone | IRS History |
|---|---|---|---|---|---|---|
| Mopti | Mopti | Tongorongo | Sarema | Sprayed | Sahelian | OP 2017, SS 2018, OP 2019 |
| | Bandiagara | Bandiagara central | Dandoly | Sprayed | | OP 2017, 2018, 2019 |
| | Djenné | Madiama | Madiama | Sprayed | Sahelian Flooded | OP 2017, SS 2018, SS 2019 |
| Segou | Tominian | Ouan | Ouena | Unsprayed | Sahelian | Unsprayed 2017-19 |

**Note: OP = organophosphate (Actellic 300CS), SS = SumiShield 50WG (clothianidin)**

**Figure 2. Average monthly temperature and rainfall for 1991-2016 for Mopti District, used for longitudinal entomological monitoring by HLC and PSC since 2017.**




Source: Climatic Research Unit (CRU) of University of East Anglia (UEA).

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 = total number of *An. gambiae* s.l. collected per month / total number of trap nights per month.

### 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. During each night of HLC, two collectors, each equipped with a mouth aspirator and a flashlight sat in each house and rotated position every hour: one indoors (living room) and the second outdoors (within 2m of the house). The following morning, mosquito identification was performed using the morphological keys of Gillies and Coetzee (1987).

### 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 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. Ten to fifteen minutes after the spraying of houses, the knocked-down mosquitoes were collected from the white canvas sheets previously laid down on the floor and furniture of the houses. Mosquitoes were put in labeled petri dishes and were later morphologically identified to genus (Anophelinae or Culicinae) then separated by sex. All female *Anopheles* were assessed for their abdominal status (unfed, fed, half gravid and gravid) and identified to species. Female *An. gambiae* s.l. and *An. funestus* were stored in 1.5 ml labeled Eppendorf tubes containing 70% ethanol for further molecular laboratory analyses.

PSC results are presented as the mean indoor resting density per house according to the following calculation: Mean indoor resting density per month = total number of *An. gambiae* s.l. collected by PSC per month / total number of houses surveyed 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 2019 through February 2020 (testing will continue until mortality is less than 80% for two consecutive months). Cone bioassays were conducted in a total of 20 structures (10 in Djenné where SumiShield 50WG was sprayed and 5 each in Mopti and Bandiagara where Actellic 300CS was sprayed) 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. Negative controls consisted of at least 30 female *An. coluzzii* tested in parallel on an unsprayed wall in each site.

The contribution of airborne effects to overall mortality in cone bioassays was also assessed using fumigant bioassays. Thus, 10-12 females *An. coluzzii* susceptible strain were introduced into a cage set on a chair,

approximately 10 cm away from the sprayed wall and about 1m above the floor. After 30 mins exposure, subsequent mortality was recorded 24 hours later for Actellic 300CS and every 24 hours for seven consecutive days for SumiShield 50WG.

## 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 and PSC 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 = (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 2019, *Anopheles* larvae were collected by the VectorLink Mali team in all 13 insecticide resistance monitoring sentinel sites and reared to adults for insecticide susceptibility tests. Four batches of 20 to 25 non-blood-fed female *Anopheles gambiae* s.l, aged two to five days, were used for the susceptibility tests according to the WHO protocol (WHO, 2016). These tests were performed with the diagnostic concentration of permethrin, alpha-cypermethrin, deltamethrin and pirimiphos-methyl. Knock-down was recorded at 60 minutes. After 60 minutes of exposure, the mosquitoes were transferred into observation tubes and fed with a 10% sugar solution. Furthermore, susceptibility to clothianidin (2% filter papers) using wild *An. gambiae* s.l. from three IRS sites was assessed using tube tests. 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. Clothianidin tests were conducted using filter papers prepared by VectorLink staff. A solution was prepared using 264mg SumiShield 50WG dissolved in 20ml distilled water. Whatman® No.1 filter papers were treated using a pipette to dispense 2ml of solution on each 12 by 15cm filter paper, resulting in a concentration of 13.2mg active ingredient clothianidin per 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.

### 2.5.2 CDC BOTTLE BIOASSAYS

Chlorfenapyr was tested using CDC bottle bioassays at 50µg, 100µg (interim diagnostic dose) and 200µg, per bottle. An initial stock solution was obtained after dilution of 100mg chlorfenapyr in 100ml acetone. Dilutions were made from this solution (stock) to have the different concentrations above. Both the susceptible strain and the field strain were tested at the same time for each site. All the chlorfenapyr tests were performed in the VectorLink bioassay room in Bamako. The exposure time was 30 minutes, with mortality recorded daily for up 3 days in order 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 by mouth aspirator to a different WHO tube with a pyrethroid-treated paper for a further 60 minutes. All WHO bioassays were conducted with 2-5 day-old *An. gambiae* s.l. reared from collected larvae. At least 100 mosquitoes were exposed to the PBO synergist at a time in four replicates of 25 mosquitoes each before being exposed to permethrin, deltamethrin or alpha-cypermethrin. There were two control tests, one involving exposure of 25 mosquitoes to 4% PBO and the other being 25 mosquitoes exposed to a solvent treated filter paper. After exposure, all mosquitoes were transferred to a holding tube and provided with 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 ace1R). Identification of *Anopheles 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 L1014F and L1014S kdr 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).

# 3. RESULTS

## 3.1 MALARIA VECTOR SPECIES COMPOSITION

Over the study period of May to December 2019, a total of 5,498 mosquitoes were collected from four sites through HLC, of which 26% were *An. gambiae* s.l. and 74% were Culicine mosquitoes. During the same period a total of 9,046 *Anopheles* were collected by PSC, of which 98% were *An. gambiae* s.l. After combining HLC and PSC data, *Anopheles* mosquitoes accounted for 56% (10,481/18,663) of the total catch. Anopheline species composition by HLC and PSC for all sites combined is shown in Figure 3. Figure 4 shows the breakdown of Anopheline species composition by site for both collection methods combined. Among the four different *Anopheles* species collected (*An. gambiae* s.l., *An. funestus* s.l., *An. pharoensis*, and *An. rufipes*), *An. gambiae* s.l. was the most predominant species accounted for 98.5% of *Anopheles* collected (10,324/10,481).

**Figure 3. Morphological species composition for all *Anopheles* collected by human landing and pyrethrum spray catches in all four sites.**

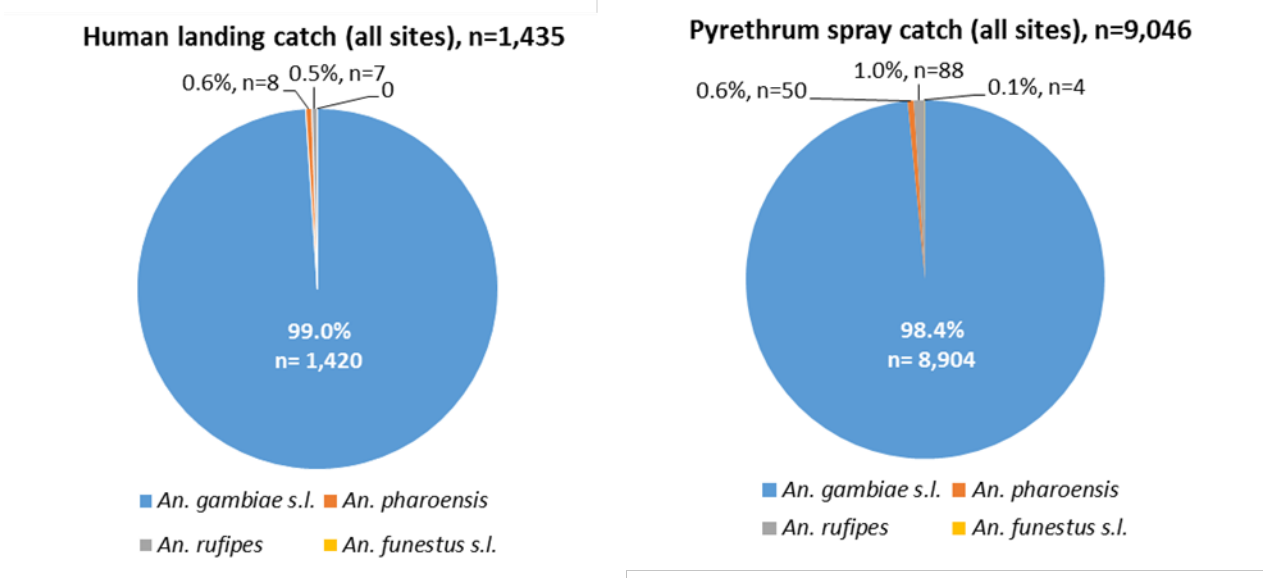
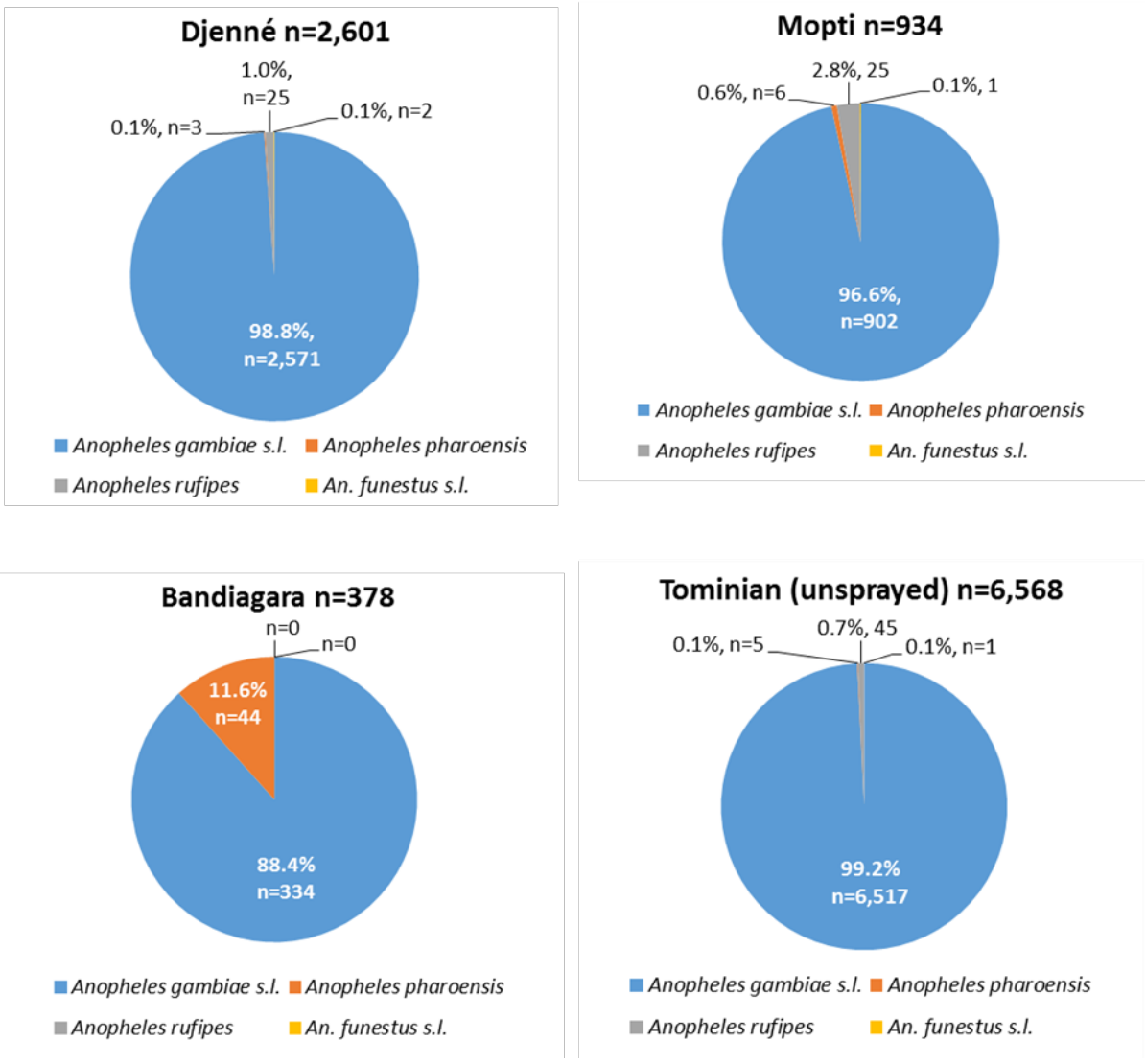


**Figure 4. Morphological species composition for all *Anopheles* collected by human landing catch and pyrethrum catch in three IRS sites (Bandiagara, Mopti and Djenné) and the unsprayed control site (Tominian) from May to December, 2019.**

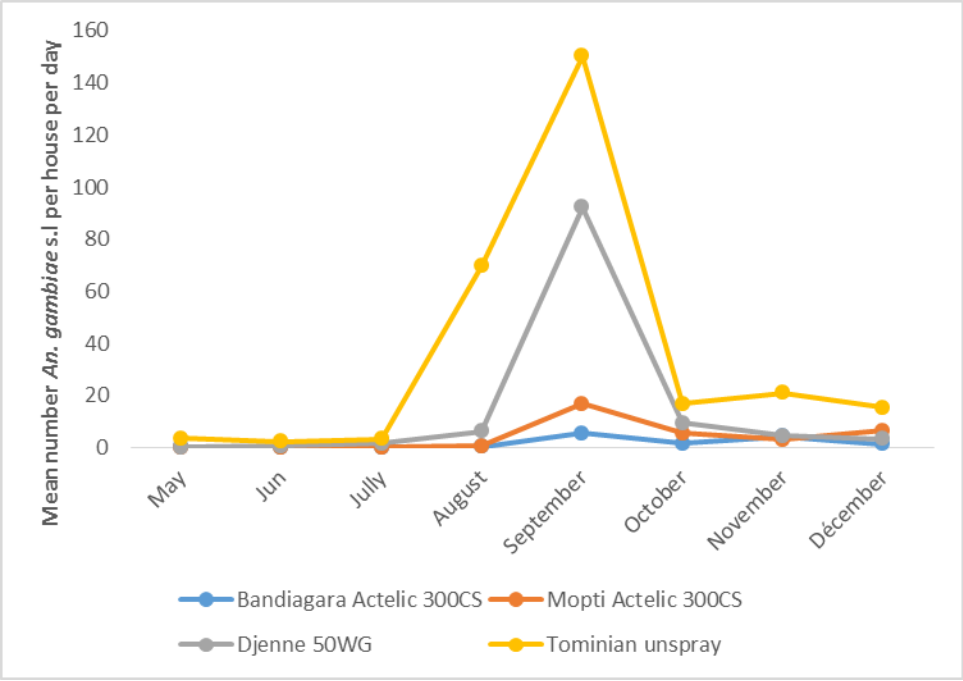



## 3.2 MALARIA VECTOR INDOOR RESTING DENSITY (BY PSC)

Figure 5 presents monthly PSC data for three sprayed sites in Mopti Region and one unsprayed site in neighboring Segou Region. The indoor vector density was very low, with a mean of less than 2 *An. gambiae* s.l. per house per day, in all sites in Mopti Region and the control sites of Tominian during the pre-spray period of June and July. In August the indoor resting densities remained low in Mopti and Bandiagara sites sprayed with Actelic 300CS. In Djenné, sprayed with SumiShield 50WG, there was a modest increase (6*An. gambiae* s.l. per house per day) compared to the unsprayed site, with significant increase of more than 70 *An. gambiae* s.l. per house per day. The peak of indoor resting densities was observed in September in all sites. The control site of Tominian had an extremely high density of 150 *An. gambiae* s.l. per house per day, while Djenné had

the highest density among the sprayed sites at 92 *An. gambiae* s.l. per house per day. Densities were lower in Mopti, with 16 *An. gambiae* s.l. per house per day and 5 in Bandiagara during the peak in September. The overall density decreased progressively between October and December in all sites, but was relatively higher in the control site of Tominian than the sprayed sites.

**Figure 5. Mean indoor resting density per house per day of *An. gambiae* s.l. captured by PSC in three IRS sites and one unsprayed control site from monthly collections between May and December 2019.**




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

The abdominal status of *An. gambiae* s.l. collected by PSC is presented for each site in Table 2. The percentage of gravid *An. gambiae* s.l. was highest in unsprayed Tominian (32%), and was lowest in sites sprayed with Actellic CS (Mopti 19% and Bandiagara 14%). The gravid proportion in Djenné (28%) was similar to the proportion in the unsprayed site, possibly due to the slower action of SumiShield WG. The proportion of half-gravid and gravid *An. gambiae* s.l. was slightly greater than unfed and blood-fed in Tominian. There was a significant difference ($X^2 = 44,6$ ; $p<0.0001$) between the proportions of unfed+fed compared to half gravid+gravid *An gambiae* s.l between Djenné and its control site Tominian .

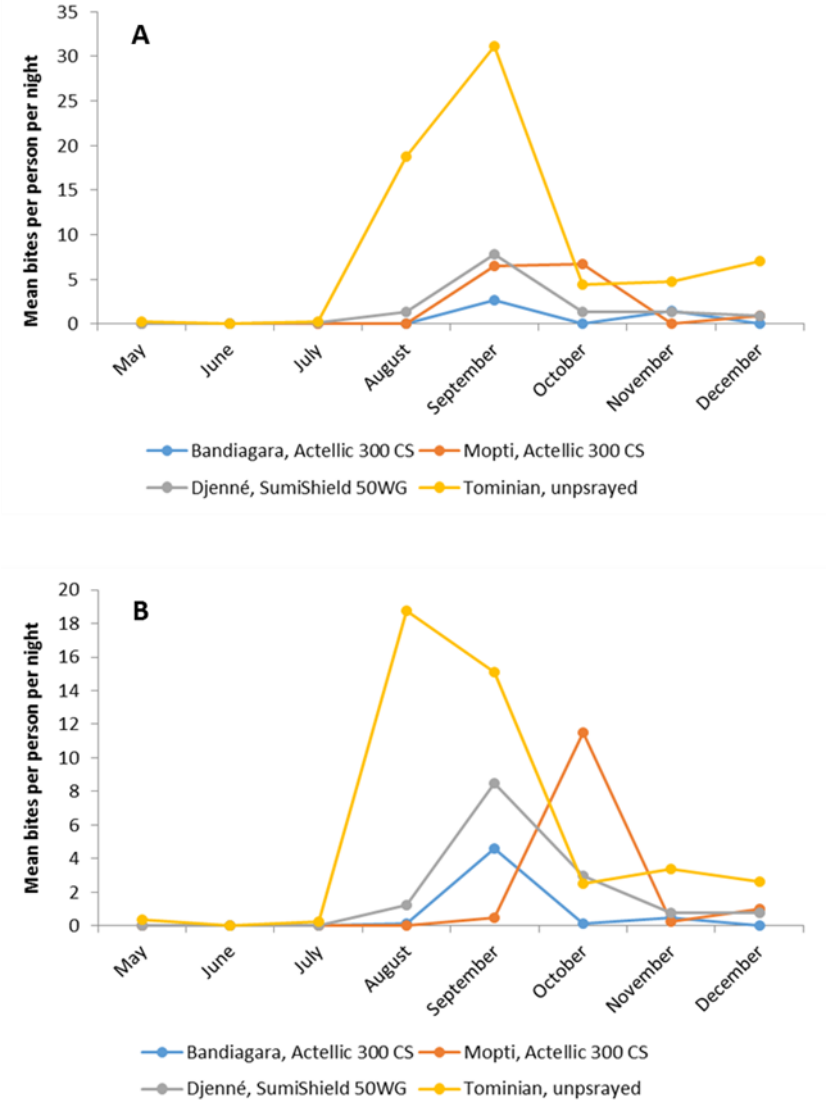
**Table 2. Abdominal status of *An. gambiae* s.l. collected by PSC form May to December, 2019.**

| Site and insecticide sprayed | Unfed N (%) | Fed N (%) | Half gravid N (%) | Gravid N (%) | Total |
|---|---|---|---|---|---|
| Bandiagara, Actellic CS | 14 (5%) | 124 (47%) | 90 (34%) | 36 (14%) | 264 |
| Mopti, Actellic CS | 41 (6%) | 237 (37%) | 242 (37%) | 125 (19%) | 645 |
| Djenné, SumiShield WG | 111 (5%) | 961 (41%) | 624 (26%) | 658 (28%) | 2,354 |
| Tominian, Unsprayed | 816 (14%) | 1454 (26%) | 1,539 (27%) | 1,832 (32%) | 5,641 |

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

During the end of the dry season (May to June) the human biting rate was zero in all IRS sites. In the unsprayed site, it was zero in June and very low indoors and outdoors (0.2-0.3) in May. In Bandiagara (sprayed with Actellic 300CS), the average biting rate per person per night (b/p/n) was low during the rainy season and post-IRS. The peak biting rate (Fig 6) of 2.6 b/p/n indoors and 4.6 b/p/n outdoors was observed in September but biting rates were generally low and remained below 5 b/p/n in Bandiagara. In Mopti (also sprayed with Actellic 300CS) the peak biting rate was observed in October with a relatively higher outdoor biting rate (11 b/p/n) than indoor (7 b/p/n). In Djenné (sprayed with SumiShield 50WG) the biting peak was observed in September with a biting rate under 9 b/p/n both indoors and outdoors. In Tominian (unsprayed control site) the peak biting rate was observed in August and September and was considerably high than all sprayed sites with peaks of 31 b/p/n indoors in September and 19 b/p/n outdoors in August. Overall, the human biting rate was relatively low in all the three IRS districts, regardless of the insecticide sprayed but was high in the unsprayed site during the rainy season (Figures 6 and 7). Human biting rates were generally far lower than the mean indoor resting densities from PSC. This may be partly due to the small sample size by HLC (only 8 houses by HLC compared to 20 by PSC) which could have resulted in relatively unproductive houses being sampled. A limitation is that due to security issues night time supervision of HLC could not be conducted.

**Figure 6. Monthly indoor (A) and outdoor (B) biting rates of *An. gambiae* s.l. in sprayed (Mopti Region) and unsprayed control site (Segou Region) (May to December, 2019).**




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

Figures 7 to 9 show the mean biting times of *An. gambiae* s.l. as recorded by HLC indoors and outdoors between 6:00 pm and 8:00 am for Mopti, Djenné and Tominian (trends are not displayed for Bandiagara due to the low numbers collected). Biting trends were similar at all sites, with biting rates gradually increasing over the first half of the night and reaching a peak during the second half of the night between midnight and 5:00 am. In 2018, biting rates were still high during the last hour of collection between 5:00 am and 6:00 am, therefore in 2019 HLC was extended up to 8:00 am, by which time *An. gambiae* s.l. biting rates were low.
In the IRS sites, the biting rate was similar indoors and outdoors. However in the unsprayed site of Tominian, the biting rate was slightly higher indoors than outdoors.

**Figure 7. Mean *An. gambiae* s.l. hourly biting rates in Mopti (sprayed with Actellic 300CS), May to December 2019 (n=113 indoors, n=144 outdoors).**

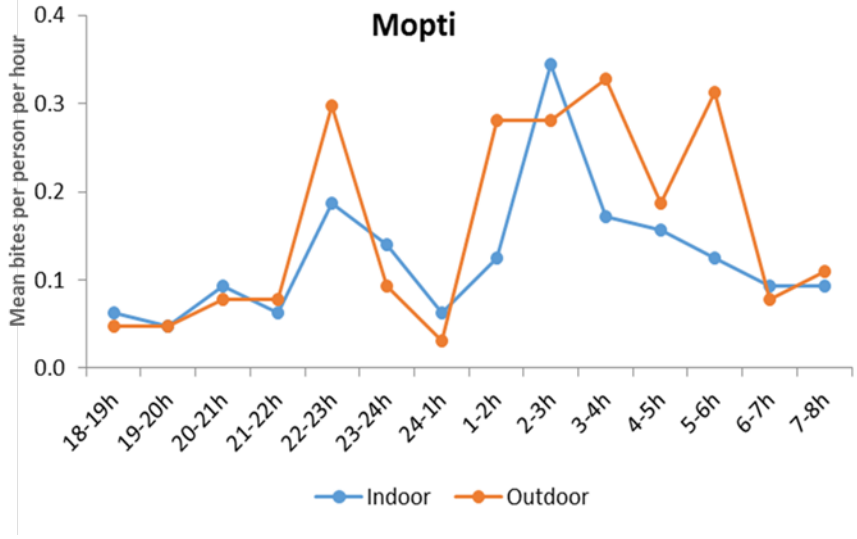



Figure 8. Mean *An. gambiae* s.l. hourly biting rates in Djenné (sprayed with SumiShield 50WG), May to December 2019 (n= indoors, n= outdoors).

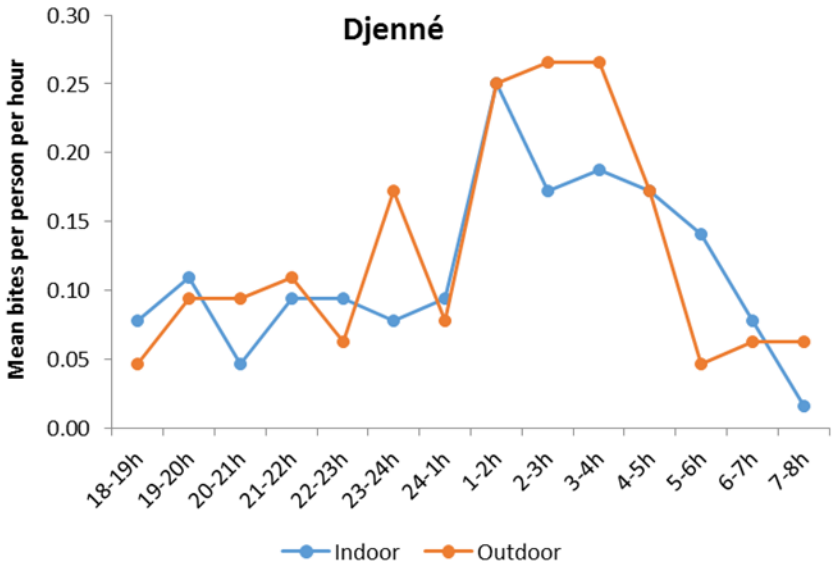
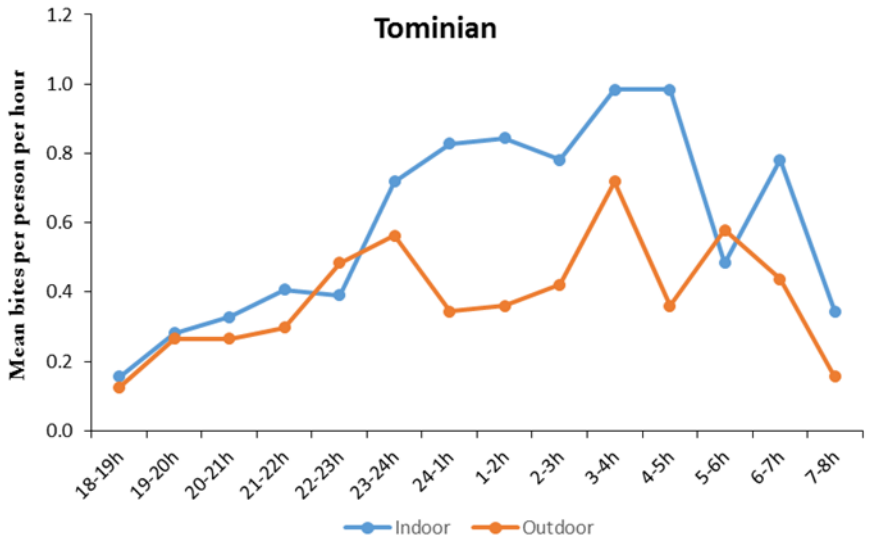


**Figure 9. Mean *An. gambiae* s.l. hourly biting rates in Tominian (Unsprayed), May-December 2019 (n=532 indoors, n=344 outdoors).**




## 3.6 *PLASMODIUM* SPOROZOITE RATE

The ELISA CSP results show the sporozoite rates are lower in all IRS sites than in the unsprayed site of Tominian. Results in table 3 are presented separately for *An. gambiae* s.l. from HLC and by PSC. The combined sporozoite rate was loweer in Mopti and Bandiagara (sprayed with Actellic 300CS) with 0.3% and 0.1% respectively compared with 1.1% in Djenné (sprayed with SumiShield 50WG) and 2.4% in Tominian (unsprayed).

**Table 3. *Anopheles gambiae* s.l *P. falciparum* sporozoite rate per site from May to December 2019.**

| Site | Total *An. gambiae* s.l. tested/total collected by HLC) | % mean sporozoite rate (positive/ tested) | Total *An. gambiae* s.l. tested/total collected by PSC | % sporozoite rate (positive/ tested) | Overall % sporozoite rate (positive/tested) |
|---|---|---|---|---|---|
| Bandiagara (Actellic 300 CS) | 61/70 | 0% (0/61) | 251/264 | 0.4% (1/251) | 0.3% (1/312) |
| Mopti (Actellic 300 CS) | 211/257 | 0.5% (1/211) | 586/645 | 0% (0/586) | 0.1% (1/797) |
| Djenné (SumiShield 50WG) | 190/217 | 1.1% (2/190) | 615/2,354 | 1.1% (7/615) | 1.1% (9/805) |
| Tominian (Unsprayed control) | 440/876 | 3.9% (17/440) | 695/5,641 | 1.4% (10/695) | 2.4% (27/1,135) |

## 3.7 ENTOMOLOGICAL INOCULATION RATE (EIR)

The entomological inoculation rate was calculated by multiplying the mean human biting rate from HLC per night by the mean sporozoite rate and multiplying by the number of days during the monitoring period (May to December = 245 days). Results are presented in Table 4 using sporozoite rates calculated from *An. gambiae* s.l. collected by HLC. As the number of *An. gambiae* s.l. was low from HLC in sprayed sites, an alternative calculation for EIR was also included using the sporozoite rate from a larger sample size using *An. gambiae* s.l. collected by HLC and PSC (bottom of table 4). In all IRS sites the EIR was relatively low <5 infective bites per person between May and December. In the unsprayed site of Tominian, the malaria risk was far greater, with an estimated EIR of 40-65 infective bites per person between May and December (depending on sporozoite rate used). As there was no supervision of HLC, it is possible that the EIR values are an underestimate of the true malaria risk, particularly as PSC catch was far greater than by HLC.

**Table 4. *An. gambiae* s.l. entomological inoculation rate (May to December 2019) for three IRS sites and one unsprayed control site.**

| | Bandiagara (Actellic 300 CS) | Mopti (Actellic 300CS) | Djenné (SumiShield 50WG) | Tominian (unsprayed control) |
|---|---|---|---|---|
| Total *An. gambiae* s.l. collected (HLC) | 70 | 257 | 217 | 876 |
| HLC trap-nights (indoors + outdoors) | 128 | 128 | 128 | 128 |
| HBR per night | 0.547 | 2.01 | 1.70 | 6.84 |
| Total *An. gambiae* s.l. tested by ELISA (HLC) | 61 | 211 | 190 | 440 |
| Sporozoite rate | 0% | 0.5% | 1.1% | 3.9% |
| EIR per night | 0 | 0.00952 | 0.0178 | 0.264 |
| **EIR May to December 2019** (using sporozoite rate from HLC samples) | **0** | **2.33** | **4.37** | **64.78** |
| EIR using sporozoite rate calculated using *An. gambiae* s.l. from HLC + PSC | | | | |
| Total *An. gambiae* s.l. tested by ELISA (HLC +PSC) | 312 | 797 | 805 | 1,135 |
| Sporozoite rate | 0.3% | 0.1% | 1.1% | 2.4% |
| EIR per night | 0.00175 | 0.00252 | 0.0190 | 0.163 |
| **EIR May to December 2019** (using sporozoite rate from HLC +PSC samples) | **0.43** | **0.62** | **4.64** | **39.89** |

## 3.8 RESIDUAL DURATION OF INSECTICIDE FORMULATIONS

### 3.8.1 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 or SumiShield 50WG on wall surfaces (mud, painted mud, cement and painted cement) tested at all sites (Figures 10-13). In Bandiagara (sprayed

with Actellic 300CS), the mortality rate decreased below 100% at T2 (two months after spraying) but remained stable just above 80% until T7 on mud walls and T6 on cement walls (Figure 10). In Mopti site (sprayed with Actellic 300CS), the mortality rate remained above 80% for ufour months on mud surfaces. However, on painted mud surfaces, the mortality rate stayed above 80% for 7 months, although there was fluctuation, with less than 80% mortality after the 6th month. In Djenné (sprayed with SumiShield 50WG), mortality is presented 24 hours (Figure 12) and 72 hours after exposure (Figure 13). Mortality was recorded up to a maximum of seven days, although in most months there was high control mortality after 72 hours holding period, therefore data beyond 72 hours is not presented. Percentage mortality on SumiShield sprayed walls was over 80% for five months on mud and painted mud, but only four months on painted cement using 24-hour mortality. Using 72-hour data, mortality was greater than 80% on all surfaces for seven months. Cone bioassay recently conducted in March (after the 8th month) but will be suspended in April due to PMI and NMCP guidance related to the COVID-19 pandemic..

**Figure 10. Percentage mortality (24h) from cone bioassay with insectary reared *An. coluzzii* tested on walls sprayed with Actellic 300CS in Bandiagara (Mopti Region).**

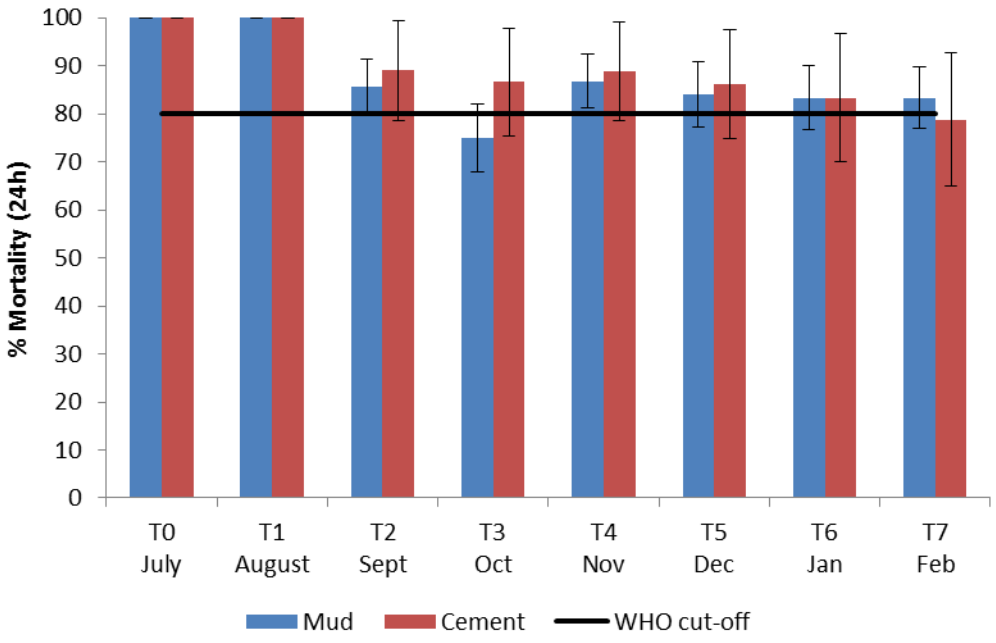


**Figure 11. Percentage mortality (24h) from cone bioassay with insectary reared *An. coluzzii* tested on walls sprayed with Actellic 300CS in Mopti (Mopti Region).**

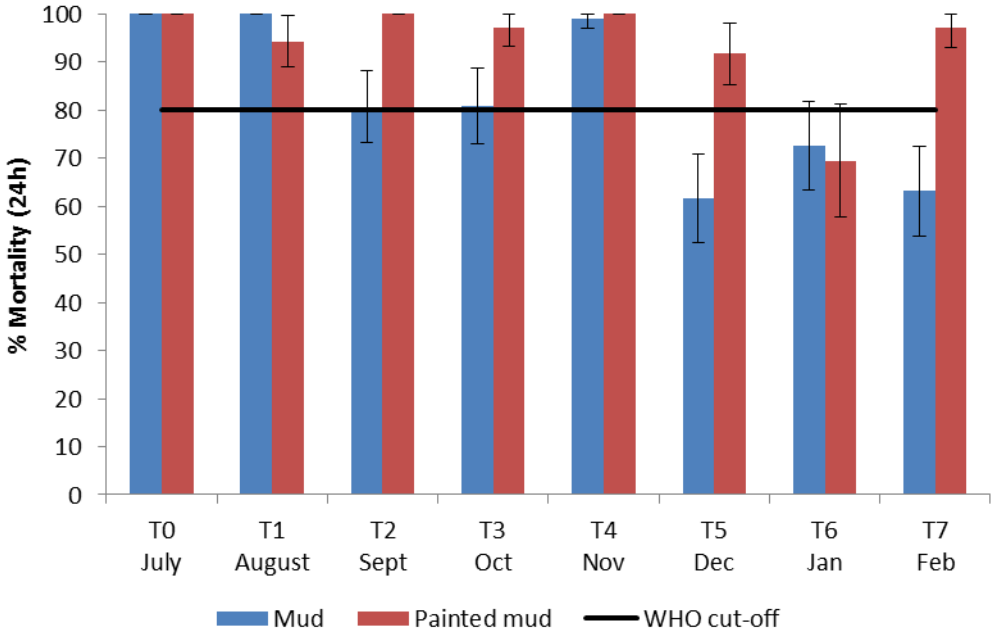



**Figure 12. Percentage mortality (24h) from cone bioassay with insectary reared *An. coluzzii* tested on walls sprayed with SumiShield 50WG in Djenné (Mopti Region).**

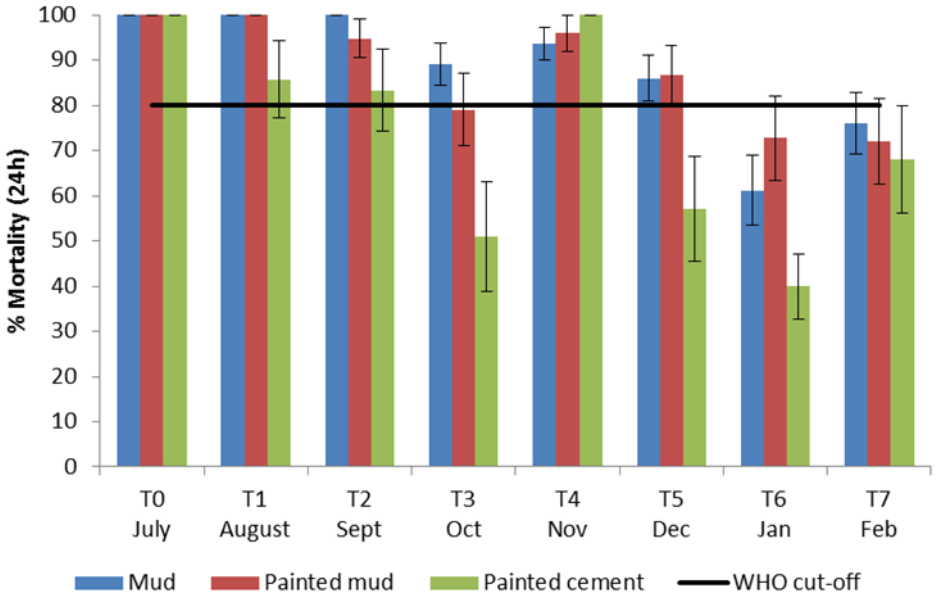
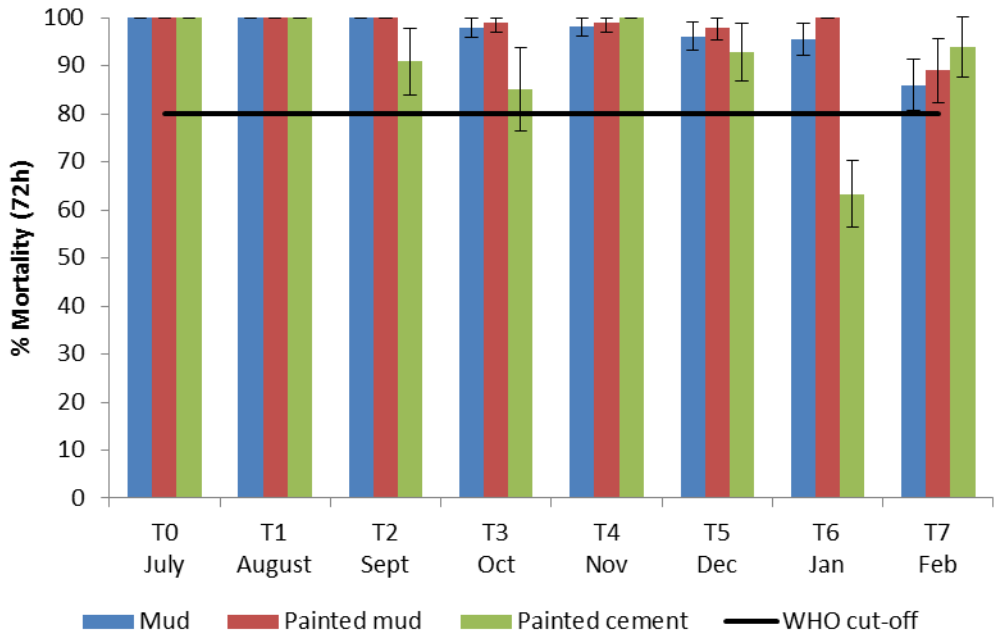


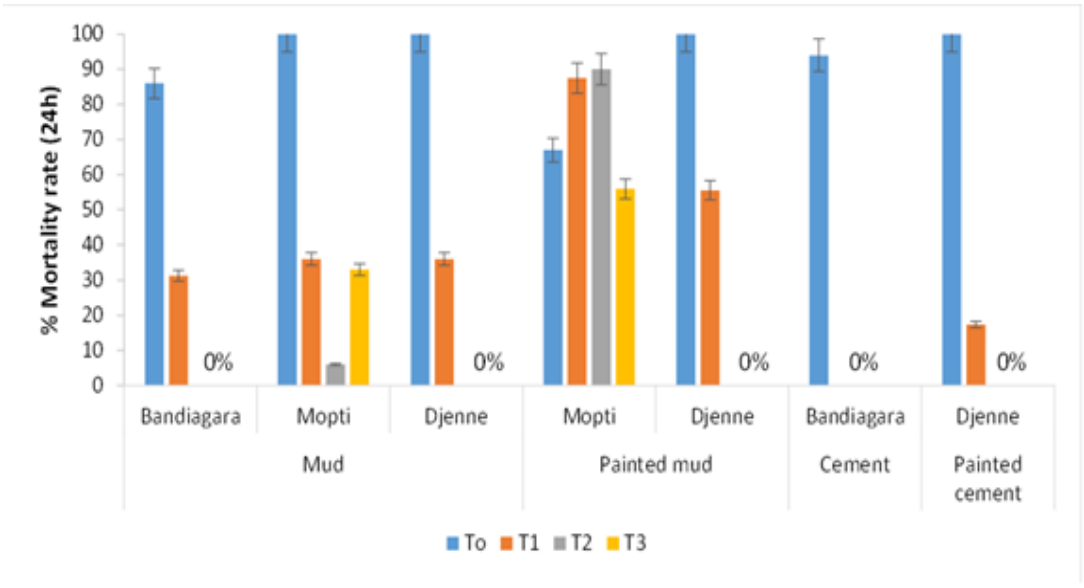
**Figure 13. Percentage mortality (72h) from cone bioassay with insectary reared *An. coluzzii* tested on walls sprayed with SumiShield 50WG in Djenné (Mopti Region).**




### 3.8.2 FUMIGANT EFFECT

The mortality rate due to the airborne effect was very high one week after IRS (T0) for both Actellic 300CS and SumiShield 50WG but decreased one month after (T1) to 0% on cement walls in Bandiagara and up to 87.5% on painted mud in Mopti. 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 others sites, the fumigant effect was short-lived and the mortality rate was less than 50% after 2-3 months (Figure 14).

**Figure 14. Monthly fumigant effect of Actellic 300CS in Mopti and Bandiagara (24h holding period) and SumiShield 50WG in Djenné (up to 120h holding period) with *An. coluzzii* (insectary strain).**



## 3.9 INSECTICIDE RESISTANCE MONITORING

### 3.9.1 SUSCEPTIBILITY OF *AN. GAMBIAE* S.L. TO PYRETHROID INSECTICIDES

According to WHO criteria, *An. gambiae* s.l. populations were resistant to the three pyrethroid insecticides (permethrin 0.75%, deltamethrin 0.05%, and alpha-cypermethrin 0.05%) tested in 2019 (Table 5) (except for Koulikoro and Kati where possible resistance was recorded for permethrin and deltamethrin respectively). Overall, mortality rates in permethrin ranged from 3% in Bla to 94% in Koulikoro, 0% (Bla) to 35% (Kita) for alpha-cypermethrin; and 0% in Koulikoro to 97% in Kati for deltamethrin. The mean mortality rates across all sites were 32% for permethrin, 29% for deltamethrin and 12% for alpha-cypermethrin. These results were consistent with those of 2018, indicating that pyrethroid resistance is stable in the sites surveyed. Pyrethroid resistance intensity was not tested in 2019 (not in the work plan) as previous annual testing for 3 years between 2016-2018 consistently showed high intensity resistance in all sites.

**Table 5. % Mortality (24h) of *An. gambiae* s.l. tested with permethrin 0.75%, deltamethin 0.05%, and alpha-cypermethrin 0.05% using WHO susceptibility tests in 2019 (compared with 2018 data); n≈100 per test.**

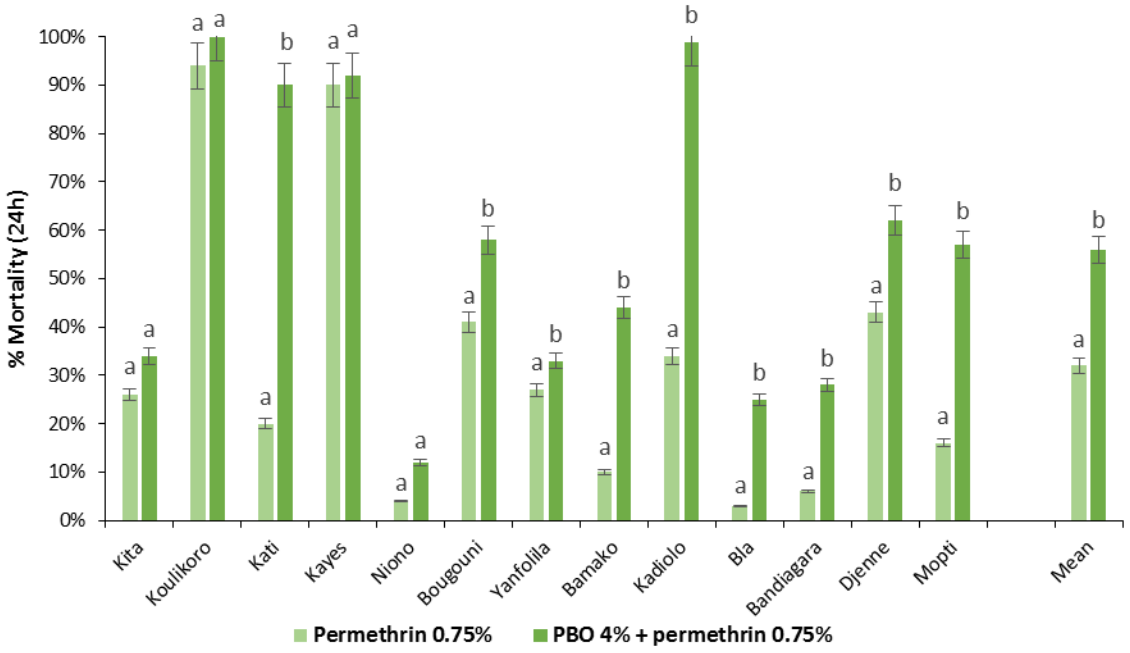
| | Permethrin 0.75% | | Deltamethrin 0.05% | | Alpha-cypermethrin 0.05% | |
|---|---|---|---|---|---|---|
| District (site) | 2018 | 2019 | 2018 | 2019 | 2018 | 2019 |
| Kita | 24% | 26% | 23% | 18% | 36% | 35% |
| Koulikoro | 16% | **94%** | 33% | 0% | 10% | 1% |
| Kati | 39% | 20% | 48% | **97%** | 10% | 4% |
| Kayes | 65% | 90% | 77% | 67% | 79% | 27% |
| Niono | 39% | 4% | 33% | 6% | 20% | 4% |
| Bougouni | 71% | 41% | 78% | 47% | 28% | 22% |
| Yanfolila | 53% | 27% | 67% | 18% | 36% | 2% |
| Bamako | 8% | 10% | 46% | 8% | 8% | 17% |
| Kadiolo | 19% | 34% | 21% | 50% | 24% | 16% |
| Bla | 8% | 3% | 61% | 2% | 7% | 0% |
| Bandiagara | 29% | 6% | 41% | 7% | 47% | 3% |
| Bankass | 32% | n/a | 70% | n/a | 20% | n/a |
| Djenne | 39% | 43% | 45% | 28% | 12% | 13% |
| Mopti | 29% | 16% | 46% | 23% | 10% | 5% |
| **MEAN** | **34%** | **32%** | **49%** | **29%** | **25%** | **12%** |

**n/a: no data available. The control mortality for each test was <5%.**

### 3.9.2 PBO SYNERGIST-INSECTICIDE BIOASSAYS

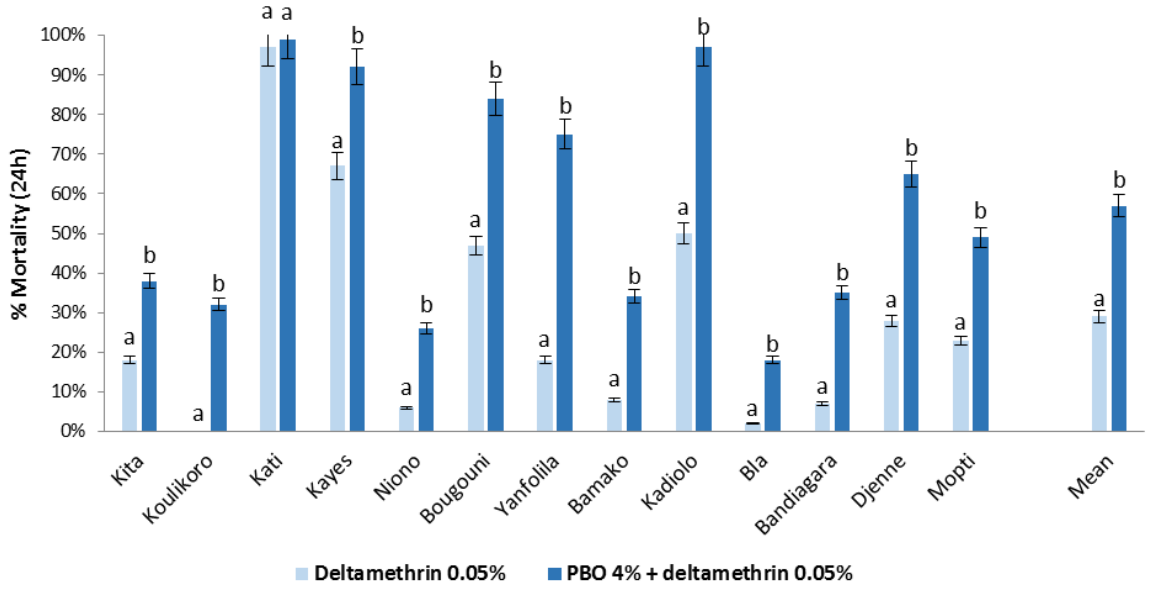
Figures 15 to 17 show the percentage mortality of *An. gambiae* s.l. exposed in tube tests to permethrin, deltamethrin and alpha-cypermethrin with and without pre-exposure to PBO. Pre-exposure to PBO generally resulted in increased mortality, but the degree varied by site and insecticide. There was a significant increase in mortality when testing with PBO + permethrin compared to permethrin only in 9 of 13 sites, 12 of 13 for PBO + deltamethrin compared to deltamethrin only, and PBO + alpha-cypermethrin in 12 of 13 sites compared to alpha-cypermethrin only. The general increase in mortality using PBO showed that mixed function oxidases are implicated in phenotypic resistance in all sites. Pre-exposure with PBO followed by permethrin resulted in 100% mortality in Kadiolo and Koulikoro and in Kati for deltamethrin, while mortality was >90% for PBO + permethrin in Kati and Kayes, Kayes and Kadiolo for deltamethrin and Kadiolo for alpha-cypermethrin. However, such high mortality rates were rare and generally mortality was below 90% despite pre-exposure with PBO. Overall the mean mortality across all 13 sites was 32% with permethrin only compared to 56% in PBO + permethrin (Figure 15), 29% for deltamethrin only compared to 57% in PBO + deltamethrin (Figure 16) and 11% for alpha-cypermethrin only compared with 47% for PBO + alpha-cypermethrin (Figure 17).

**Figure 15. % Mortality (24h) of *An. gambiae* s.l. tested with permethrin 0.75% and 4% PBO synergist + permethrin 0.75%.**



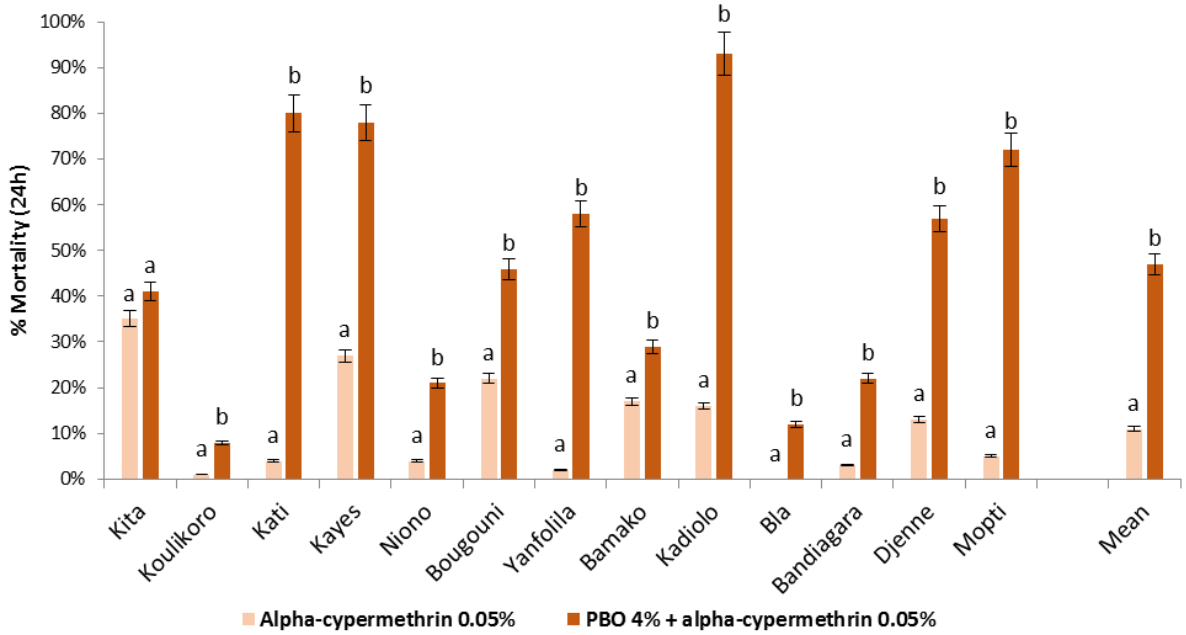

**Superscript indicates whether % mortality for permethrin is significantly different to % mortality for permethrin + PBO.**
**a = no significant difference P>0.05; b = significant difference P<0.05**

**Figure 16. % 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 17. % 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

### 3.9.3 SUSCEPTIBILITY OF *AN. GAMBIAE* S.L. TO CHLORFENAPYR

As WHO has not yet developed a guidance on susceptibility testing of chlorfenapyr, three doses (50, 100 and 200µg/bottle) were tested. An insectary strain of *An. coluzzii* was always tested in parallel to show whether mortality below 98% in the wild population was due to resistance or due to the dosage tested. Both wild *An. gambiae* s.l and the insectary strain of *An. coluzzii* showed full susceptibility to chlorfenapyr at the dose of 200µg/bottle in all 10 sites. Using the lower dosages (50µg and 100µg concentrations), mortality rates after 3 days post exposure indicated possible resistance. Resistance is unlikely as pyrrole insecticides have not been used for agriculture or public health in Mali, therefore it is most likely that these doses were too low and that 200µg/bottle is a more suitable diagnostic concentration for chlorfenapyr.

**Table 6. % Mortality (24h, 48h, 72h) of wild An. gambiae s.l. and susceptible insectary An. coluzzii Ngousso strain tested with chlorfenapyr (50µg, 100µg and 200µg/bottle) for 30 minutes exposure in CDC bottle bioassays.**

| Sites | Mosquito origin | 50µg ai/bottle | | | 100µg ai/bottle | | | 200µg ai/bottle | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 24h | 48h | 72h | 24h | 48h | 72h | 24h | 48h | 72h |
| Kita | Insectary strain | 87% | 95% | 98% | 80% | 92% | 98% | 84% | 96% | 100% |
| | Wild | 67% | 89% | 97% | 75% | 88% | 96% | 81% | 93% | 100% |
| Koulikoro | Insectary strain | 75% | 91% | 98% | 78% | 84% | 94% | 80% | 99% | 100% |
| | Wild | 73% | 87% | 95% | 55% | 71% | 89% | 88% | 93% | 100% |
| Kati | Insectary strain | 55% | 79% | 93% | 85% | 92% | 98% | 91% | 99% | 100% |
| | Wild | 57% | 82% | 95% | 68% | 73% | 90% | 75% | 90% | 100% |
| Kayes | Insectary strain | 71% | 92% | 100% | 74% | 95% | 99% | 88% | 97% | 100% |
| | Wild | 75% | 93% | 98% | 68% | 89% | 97% | 81% | 97% | 100% |
| Niono | Insectary strain | 77% | 89% | 99% | 89% | 95% | 98% | 78% | 95% | 100% |
| | Wild | 81% | 84% | 96% | 76% | 88% | 96% | 90% | 96% | 100% |
| Yanfolila | Insectary strain | 96% | 97% | 99% | 81% | 95% | 97% | 98% | 100% | 100% |
| | Wild | 55% | 82% | 85% | 37% | 60% | 93% | 25% | 64% | 100% |
| Bamako | Insectary strain | 71% | 80% | 90% | 78% | 89% | 98% | 94% | 97% | 100% |
| | Wild | 68% | 82% | 87% | 80% | 91% | 99% | 89% | 96% | 100% |
| Kadiolo | Insectary strain | 96% | 97% | 99% | 81% | 95% | 97% | 98% | 100% | 100% |
| | Wild | 88% | 97% | 98% | 91% | 94% | 97% | 97% | 98% | 98% |
| Bla | Insectary strain | 81% | 97% | 100% | 88% | 98% | 100% | 91% | 97% | 100% |
| | Wild | 89% | 95% | 100% | 82% | 94% | 99% | 90% | 97% | 100% |
| Bandiagara | Insectary strain | 94% | 99% | 99% | 71% | 94% | 99% | 80% | 97% | 100% |
| | Wild | 75% | 93% | 94% | 83% | 95% | 97% | 81% | 93% | 96% |
| Djenné | Insectary strain | 94% | 99% | 99% | 71% | 94% | 99% | 80% | 97% | 100% |
| | Wild | 84% | 95% | 95% | 53% | 76% | 82% | 65% | 86% | 97% |
| Baguenida | Insectary strain | 62% | 88% | 94% | 88% | 93% | 100% | 79% | 95% | 100% |
| | Wild | 67% | 81% | 89% | 82% | 95% | 99% | 92% | 99% | 100% |
| **MEAN** | **Insectary strain** | **80%** | **92%** | **97%** | **80%** | **93%** | **98%** | **87%** | **97%** | **100%** |
| | **Wild** | **73%** | **88%** | **94%** | **71%** | **85%** | **95%** | **80%** | **92%** | **99%** |

### 3.9.4 SUSCEPTIBILITY OF *AN. GAMBIAE* S.L. TO PIRIMIPHOS-METHYL

In 2019, full vector susceptibility to 0.25% pirimiphos methyl was found in all the 13 sentinel sites. The mortality in the negative control was 0% in all sites. The possible vector resistance to pirimiphos methyl observed in 2018 in Yanfolila (97% mortality) was not found in 2019 .

### 3.9.5 SUSCEPTIBILITY OF *AN. GAMBIAE* S.L. TO CLOTHIANIDIN

Table 7 shows the daily mortality rates following exposure to clothianidin 2% of wild *An. gambiae* s.l. populations from the 13 sentinel sites 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. Positive control 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 misreported resistance. One hundred percent mortality was recorded with the insectary strain in all sites, confirming the quality of the treated papers. Mortality rates varied substantially and, at 24 hours post-exposure, ranged between 50% in Baguineda to 100% in Mopti and Kita for wild *An. gambiae* s.l. Mortality rates in negative controls were low (below 5%) after six days.

**Table 7. Mortality rates of *An. gambiae* s.l. (wild) and *An. coluzzii* (insectary strain), tested with clothianidin 2% (13.2 mg ai/paper).**

| Region | District | Strain | % mortality after exposure to clothianidin | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | 24h | 48h | 72h | 96h | 120h | 144h | 168h |
| Segou | Bla | Insectary strain | 89.% | 93.00% | 99.00% | 100.00% | | | |
| | | Wild | 67.% | 83.00% | 91.00% | 94.00% | 97.00% | 97.00% | 99.00% |
| | Niono | Insectary strain | 92.% | 98.00% | 100.00% | | | | |
| | | Wild | 96.% | 100.00% | 100.00% | | | | |
| Koulikoro/ Bamako | Baguineda | Insectary strain | 50.00% | 81.00% | 89.00% | 98.00% | 99.00% | 100.00% | |
| | | Wild | 75.00% | 95.00% | 98.00% | 98.00% | 99.00% | 100.00% | |
| | Koulikoro | Insectary strain | 91.00% | 100.00% | | | | | |
| | | Wild | 88.00% | 98.00% | 100.00% | | | | |
| | Bamako | Insectary strain | 54.00% | 81.00% | 88.00% | 100.00% | | | |
| | | Wild | 82.00% | 92.00% | 95.00% | 100.00% | | | |
| Sikasso | Kadiolo | Insectary strain | 78.00% | 89.00% | 95.00% | 100.00% | | | |
| | | Wild | 82.00% | 93.00% | 99.00% | 99.00% | 100.00% | | |
| | Bougouni | Insectary strain | 78.00% | 89.00% | 95.00% | 100.00% | | | |
| | | Wild | 73.00% | 93.00% | 95.00% | 97.00% | 98.00% | 99.00% | |
| | Sélingué | Insectary strain | 78.00% | 89.00% | 95.00% | 100.00% | | | |
| | | Wild | 69.00% | 84.00% | 88.00% | 97.00% | 98.00% | 98.00% | |
| Kayes | Kita | Insectary strain | 93% | 100% | | | | | |
| | | Wild | 100% | | | | | | |
| | Kayes | Insectary strain | 76% | 92% | 94% | 99% | 100% | | |
| | | Wild | 77% | 91% | 93% | 95% | 97% | 99% | 99% |

| Region | District | Strain | % mortality after exposure to clothianidin | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | 24h | 48h | 72h | 96h | 120h | 144h | 168h |
| Mopti | Mopti | Insectary strain | 77% | 88% | 95% | 95% | 100% | | |
| | | Wild | 100% | | | | | | |
| | Bandiagara | Insectary strain | 89% | 99% | 99% | 100% | | | |
| | | Wild | 99% | 100% | | | | | |
| | Djenné | Insectary strain | 74% | 90% | 94% | 100% | | | |
| | | Wild | 98% | 100% | | | | | |
| **MEAN** | | **Insectary strain** | **78%** | **91%** | **96%** | **99%** | **100%** | **100%** | **100%** |
| | | **Wild** | **85%** | **95%** | **97%** | **98%** | **99%** | **100%** | **100%** |

# 4. DISCUSSION

Effectiveness of IRS with both insecticides was evidenced by the lower *An. gambiae sl* abundance, biting rate and EIR and sporozoite levels measured in IRS versus the unsprayed control site. IRS with Actellic 300CS in Bandiagara and Mopti had good residual efficacy, providing greater than 80% mortality (the WHO cut-off) for four months in Mopti and up to seven months in Bandiagara. Cone bioassays demonstrated that SumiShield 50WG provided a minimum of seven months residual control in Djenné if measured 72 hours post-exposure mortality, but mortality was lower using 24-hour results. As the high transmission season is short in Mopti Region (lasting approximately four months and the populations showed susceptibility to pirimiphos-methyl and clothianidin, either Actellic 300CS or SumiShield 50WG can be used as part of a rotation strategy to delay the development of resistance.

The peak of indoor resting densities was observed in September in all sites. The control site of Tominian had an extremely high density of 150 *An. gambiae* s.l. per house per day, while Djenné had the highest density of the sprayed sites at 92 *An. gambiae* s.l. per house per day (only two months after IRS). Densities were lower: 16 *An. gambiae* s.l. per house per day in Mopti and 5 in Bandiagara during the peak in September. The density then decreased progressively between October and December in all sites, but remained relatively higher in the control site of Tominian.. Indoor resting densities were higher in the site sprayed with SumiShield 50WG than those sprayed with Actellic 300CS. This may be due to the more slow-acting nature of SumiShield 50WG, meaning that mosquitoes may survive long-enough to be captured by morning pyrethrum spray catch.

Human biting rates were generally far lower than the mean indoor resting densities from PSC. In Tominian the peak biting rate was observed in August and September and was significantly higher than in Djenne ($X^2$ = 4,35 ; P= 0.03), reaching 31 b/p/n indoors in September and 19 b/p/n outdoors in August. No significant difference was found between HBR in the 3 IRS sites. IRS was conducted in July and based on malaria vector densities this appears to be optimal timing shortly before the peak in malaria vector densities which was between August and October.

The overall sporozoite rate (from HLC and PSC samples) was lowest in Mopti and Bandiagara (sprayed with Actellic 300CS) at 0.3% and 0.1% compared with 1.1% in Djenné (sprayed with SumiShield 50WG) and 2.4% in Tominian (unsprayed). In all IRS sites the EIR was below 5 infectious bites per person between May and December. In Tominian, the malaria risk was far greater, with an estimated EIR of 40-65 infectious bites per person between May and December. Despite limitations including only one unsprayed site and no baseline entomological data for the IRS sites, it appears that IRS is having a positive effect in Mopti Region in terms of malaria vector biting and infectivity rates. However, as night time supervision of HLC was not possible due to insecurity, it is likely that the EIR is even higher in all sites, particularly as PSC resulted in a much

higher catch size than HLC (generally catch size is greater by HLC than PSC, as mosquitoes bite and then a proportion rest indoors).

Full susceptibility was found in all the 13 sentinel sites to pirimiphos-methyl and clothianidin including IRS sites of Mopti, Bandiagara and Djenné. Therefore, based on continuing susceptibility both Actellic 300CS (pirimiphos-methyl) and clothianidin formulations SumiShield 50 WG) can be considered for IRS as part of a rotation strategy. *An. gambiae* s.l. were resistant to the three pyrethroid insecticides that are commonly used on ITNs (permethrin, deltamethrin and alpha-cypermethrin). PBO synergist tests show that mixed function oxidases (MFOs) are implicated in pyrethroid resistance. Significantly increased mortality rates were obtained in all sites with these three insecticides after pre-exposure of *An. gambiae* s.l. to PBO, although mortality was generally still below 90%. Wild *An. gambiae* s.l from all 10 sites showed susceptibility to chlorfenapyr in bottle bioassays at a dose of 200µg/bottle. Due to the widespread presence of pyrethroid resistance and limited impact of PBO synergists, next generation ITNs such as Interceptor G2 (containing chlorfenapyr + pyrethroid) should be prioritized for future ITN distribution campaigns. Fortunately as part of the 'New Nets Project' 2 million Interceptor G2 nets are being distributed in several countries, including Mali in 2020 as an operational pilot to build evidence regarding the cost-effectiveness of dual active ingredient nets.