U.S. PRESIDENT'S MALARIA INITIATIVE

# PMI VECTORLINK BURKINA FASO ENTOMOLOGICAL MONITORING ANNUAL REPORT

## JANUARY – DECEMBER 2019

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**Approved on:**

**Abt Associates Inc. | 6130 Executive Boulevard**
**Rockville, Maryland 20852 | T. 301.347.5000 | F. 301.913.9061**
**abtassociates.com**

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# ACRONYMS

| | |
|---|---|
| CS | Capsule Suspension |
| EIR | Entomological Inoculation Rate |
| HBR | Human Biting Rate |
| HLC | Human Landing Catch |
| IRS | Indoor Residual Spraying |
| LLIN | Long-Lasting Insecticidal Net |
| NMCP | National Malaria Control Program |
| PBO | Piperonyl Butoxide |
| PMI | U.S. President's Malaria Initiative |
| PSC | Pyrethrum Spray Catch |
| WG | Wettable Granules |
| WG | Water Dispersible Granules |
| WHO | World Health Organization |
| WP-SB | Wettable Powder in Water Soluble Bag |

# EXECUTIVE SUMMARY

The Burkina Faso 2016-2020 National Malaria Strategic Plan recommends the use of non-pyrethroid indoor residual spray (IRS) as complementary to long lasting insecticidal nets (LLINs) used in malaria vector control in locations where pyrethroid resistance occurs. In 2019, PMI VectorLink conducted spray operations from June 6, 2019 to July 11, 2019 in three districts (Kongoussi, Solenzo and Kampti) using three insecticide formulations (Actellic CS, Fludora Fusion WP-SB and SumiShield WG). The project sprayed 201,901 out of 220,482 eligible structures, accounting for a coverage rate of 91.6 percent. In addition, a nationwide mass campaign distributed 1.5 million piperonyl butoxide (PBO)-synergist PermaNet 3.0 LLINs and two million dual-active ingredient Interceptor G2 LLINs, and standard pyrethroid LLINs between June and October 2019.

To monitor the impact of vector control on entomological indicators, monthly cone bioassay was conducted on sprayed walls, and monthly entomological surveillance using pyrethrum spray catch (PSC) and human landing catch (HLC) conducted in the sprayed sites (Kongoussi, Solenzo, Kampti). Entomological monitoring was also conducted at three unsprayed sites (Seguenega, Nouna, Gaoua), and at two sites (Karangasso-Vigué and Soumousso) where PBO LLINs were distributed. Insecticide susceptibility tests were conducted in 14 sites to determine the appropriate selection of insecticide for future IRS rounds and LLIN distribution campaigns. Susceptibility tests using *An. gambiae* s.l. were conducted with pyrethroid insecticides and PBO synergists according to World Health Organization (WHO) protocols. CDC bottle bioassays were also conducted to determine susceptibility status to the new insecticide chlorfenapyr. In addition, the team performed WHO tube tests to determine susceptibility to pirimiphos-methyl and clothianidin, both of which were used for IRS.

Cone bioassays, with a susceptible insectary strain (*Anopheles gambiae* Kisumu), showed that Actellic CS, Fludora Fusion WP-SB and SumiShield WG all lasted for at least seven months in all sites. Therefore, all three formulations can continue to be used as part of a rotation strategy to provide control during the peak malaria transmission season. *Anopheles gambiae* was the predominant malaria vector species in the Southwest (Gaoua, Kampti, Karangasso-Vigué and Soumousso), while *An. coluzzii* was more frequent in the Centre Nord (Seguenega and Kongoussi) and Centre West (Nouna and Solenzo). The proportion of *An. arabiensis* being collected in the southwestern regions appears to be gradually increasing compared to previous years (but still represents a very small proportion of malaria vector species).

The peak indoor resting densities and biting rates was observed in September/October in all sites, approximately three to four months after spraying. The mean indoor and outdoor biting rates were consistently lower in Kongoussi (Actellic CS/SumiShield WG)) compared to Seguenega (unsprayed). However, biting rates were far greater in Solenzo (SumiShield WG) than in Nouna (unsprayed), and there was no clear difference in biting rates for Kampti (Fludora Fusion WP-SB) and Gaoua (unsprayed). When broken down to sub-locations, IRS appears to have an impact in central sites compared to paired unsprayed areas. However, in rural sites where biting rates were higher, IRS had a lesser impact on entomological indices. The density of *An. gambiae* s.l. collected from PSC was generally lower in IRS sites than in the unsprayed control sites, particularly in Kongoussi and Kampti, where densities were always < 6 *An. gambiae* s.l. per house per day. In Kampti (sprayed with Fludora Fusion WP-SB), the PSC collected fewer than 3 *An. gambiae* s.l. per house/night from July to December which was statistically lower than neighboring unsprayed Gaoua (RR= 3.97 [2.65, 5.95], Tukey's p< 0.001). However, in Solenzo (SumiShield WG), the densities were not significantly different from the paired unsprayed site of Nouna and reached a peak mean of 17 *An. gambiae* s.l. per day in October, four months after IRS application.

Parity rates showed reduced proportions of parous *An. gambiae* s.l. in Kongoussi (Actellic CS/SumiShield WG), 63 percent) compared to Seguenega (unsprayed, 78 percent), as well as in Solenzo (SumiShield WG, 70 percent) compared to Nouna (unsprayed, 78 percent). The mean sporozoite infection rates were high and did not differ between sprayed and unsprayed sites, ranging from 8.5 percent in Seguenega (unsprayed) to 14.1 percent in Solenzo (SumiShield WG).

The entomological inoculation rate (EIR) was high in the Southwest, with Gaoua (unsprayed) with an EIR of 160 infective bites per person between June and December, 2019. The EIR was relatively lower in the neighboring district of Kampti sprayed with Fludora Fusion WP-SB, but the malaria transmission risk was still high with 111 infective bites per person. In the central sites, the EIR was significantly lower in the district of Kongoussi sprayed with Actellic CS/SumiShield WG with 66 infective bites per person compared to the unsprayed district of Seguenega with 125 infectious bites per person, with approximately two-fold lower risk in Kongoussi compared to Seguenega (RR=4.73, IC95%= [1.41–15.87], p=0.011). In Nouna (unsprayed), the EIR showed 42 infective bites per person compared to 41 infective bites per person in Solenzo (SumiShield WG). Overall, IRS appears to have an impact on EIR in two out of the three sites, but malaria continues to be a considerable risk in all the sites. IRS appears to be having some impact on malaria vector biting and resting proportions, particularly in central area; however there was less impact in rural sites where vector densities were higher. In the two sites where PBO LLINs were distributed, the EIR was high, at 102 infective bites per person in Karangasso-Vigué and 114 in Soumousso.

Insecticides resistance monitoring data showed that *An gambiae* s.l. was fully susceptible to pirimiphos-methyl and clothianidin at all sites tested, including all three IRS locations. Therefore, these insecticide formulations can continue to be used in Burkina Faso for IRS in rotation. The insecticide susceptibility tests also revealed that *An. gambiae* s.l. was resistant to all pyrethroids tested, but pre-exposure to PBO increased mosquito susceptibility to deltamethrin and permethrin at all sites tested, including Karangasso-Vigue and Soumousso, where Permanet 3.0 nets were distributed in 2019. Pyrethroid resistance intensity was high in all sites for deltamethrin and alpha-cypermethrin and mostly moderate or high for permethrin. This high resistance intensity could be undermining performance of pyrethroid only LLINs. On the other hand, *An. gambiae s.l* was susceptibile to chlorfenapyr at all sites. Due to the widespread presence of pyrethroid resistance, PBO synergists and next generation LLINs, such as Interceptor G2 (containing chlorfenapyr + pyrethroid), should continue to be prioritized for future LLIN distribution campaign.

# I. INTRODUCTION

The World Health Organization (WHO) has reported 228 million malaria cases and 405,000 deaths worldwide in 2018 (WHO, 2019). Malaria is endemic in Burkina Faso, and the recent National Malaria Control Program (NMCP) Report published in 2018 showed that malaria morbidity is still increasing, especially in children under five and pregnant women (NMCP report, 2019). In 2018, the NMCP recorded approximately 11.9 million confirmed cases of malaria and 4,292 deaths reported by health facilities in the country's overall population, classifying Burkina Faso as the third most affected country after the Democratic Republic of Congo and Nigeria (NMCP report, 2019). The primary malaria parasite is *Plasmodium falciparum* (Hien *et al*., 2017) primarily transmitted by *Anopheles gambiae* s.l. and *Anopheles funestus* (Dabiré *et al.,* 2007, 2012). The use of long-lasting insecticide treated bednets (LLIN) remains the main tool for malaria vector control in Burkina Faso. However, resistance to pyrethroids in malaria vectors has spread across Africa and a major threat to malaria control (Hemingway *et al*., 2016).

A common mechanism of resistance to pyrethroids, the knock-down resistance mutation (*kdr*-1014F), emerged in Burkina Faso toward the end of the 1990's (Chandre *et al*., 1999). This *kdr*-1014F mutation (Diabaté *et al*., 2004; Dabiré *et al*., 2012; Toé *et al*., 2015) spread quickly in Burkina Faso and broadly in West Africa, and is acting in combination with metabolic resistance mechanisms that could reduce the efficacy of pyrethroid LLINs (Toé *et al*., 2015).

The NMCP's 2016-2020 national malaria strategic plan recommends that non-pyrethroid-based IRS be used as a complementary vector control tool together with LLINs in locations where pyrethroid resistance occurs. This is partly due to the availability of new non-pyrethroid IRS formulations that can provide long-lasting control of pyrethroid resistance malaria vectors. In partnership with the PMI program, the NMCP prioritized IRS as a complementary vector control strategy to be implemented annually from 2018. In 2019, PMI VectorLink conducted spray operations from June 6, 2019 to July 11, 2019 in Kongoussi, Solenzo and Kampti districts using three insecticide formulations (Actellic CS, Fludora Fusion and Sumishield). The project sprayed 201,901 out of 220,482 eligible structures, accounting for a coverage rate of 91.6 percent. In addition, a nationwide mass distribution campaign supplied 1.5 million piperonyl butoxide (PBO)-synergist PermaNet 3.0 LLINs and two million dual-active ingredient Interceptor G2 LLINs, in addition to standard pyrethroid LLINs between June and October 2019.

The VectorLink Burkina Faso team conducted surveys to monitor vector bionomics and insecticide susceptibility during the period of high malaria transmission (June to December 2019) in three eco-climatic zones (Sudanian, Sudano-Sahelian and Sahelian) to monitor IRS sites and their respective neighbouring (unsprayed) control sites. Nationwide resistance data was also collected and shared with the NMCP to assist with vector control planning. This report provides the key results from the six-month (June-December 2019) data collection to monitor the impact of IRS on malaria control Burkina Faso.

The main objective of the entomological monitoring activities in Burkina Faso is to monitor the residual efficacy of IRS in houses sprayed with Actellic CS, Sumishield 50 WG and Fludora Fusion WP-SB and to determine the impact of IRS in 2019.

The specific objectives of the program included:

- Collect detailed information on mosquito biting rates, biting times, indoor resting densities, seasonality, and parity rates of malaria vectors in both IRS sites and their adjacent unsprayed control sites.
- Determine the impact of IRS on entomological outcomes by comparing the 2019 data from sprayed and unsprayed sites
- Conduct laboratory analysis of mosquito samples to determine vector species composition, presence of molecular markers of resistance (*kdr-w*, *kdr-e* and *Ace-1*), blood-meal source, and *P. falciparum* sporozoite infection rates.
- Determine the susceptibility level of the main malaria vectors, *Anopheles gambiae* s.l., to two new insecticides recommended, specifically clothianidin and chlofenapyr, in six PMI sites.
- Monitor the susceptibility of *An. gambiae* s.l. to permetrhin 0.75 percent, deltamethrin 0.05 percent, alphacypermthrin (with and without pre-exposure to the synergist piperonyl-butoxide (PBO)), bendiocarb 0.1 percent, and pirimiphos-methyl 0.25 percent.
- Determine the intensity of insecticide resistance to pyrethroids (permethrin, deltamethrin and alphacypermethrin), using WHO tube protocol.
- Provide technical assistance to the NMCP in the development of its national resistance monitoring plan.

# 2. METHODOLOGY

## 2.1 STUDY AREA

Monthly longitudinal entomological monitoring was carried out during the high transmission season from June to December 2019 in ten sites: three IRS sites (Kongoussi, Solenzo and Kampti), three paired unsprayed control sites located approximately 50km away (Seguenega, Nouna and Gaoua), two sites (Soumousso and Karangasso-Vigué) where PBO LLINs were distributed, and two sites (Orodara and Banfora) where Intercepter G2 LLINs were distributed for durability monitoring. Monthly mosquito collections were conducted to measure entomological parameters of malaria transmission. These sites are located across the three ecological zones of Burkina Faso: Sudan (West), Sudan-Sahelian (Centre-West) and Sahelian (North) ecological zones (Figure 1).

*Anopheles* mosquitoes were sampled between June and December 2019, during the rainy season, using three sampling methods: i) human landing catches (HLC), ii) pyrethrum spray catches (PSC), and iii) larval collections for insecticide resistance monitoring. Longitudinal trapping by HLC and PSC was done in two sub-locations within each site, in the central and rural sub-locations. Cone bioassays were conducted on sprayed walls in the three IRS districts to measure the quality of IRS and residual efficacy.

**Figure 1. Study sites including IRS intervention sites (blue) and their respective unsprayed control sites (red).**

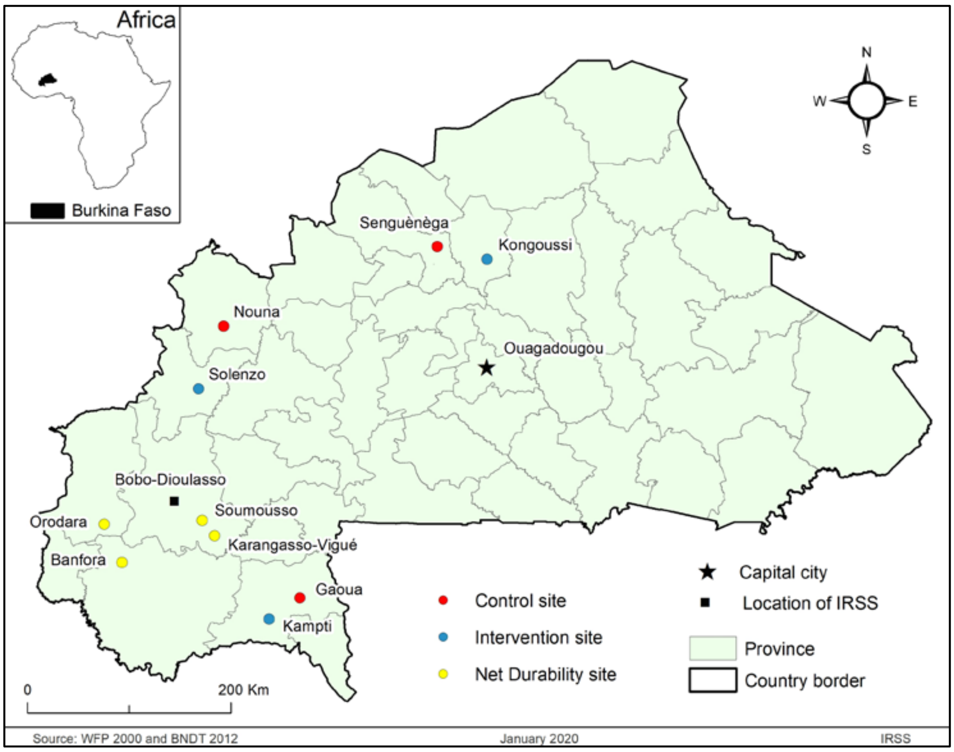
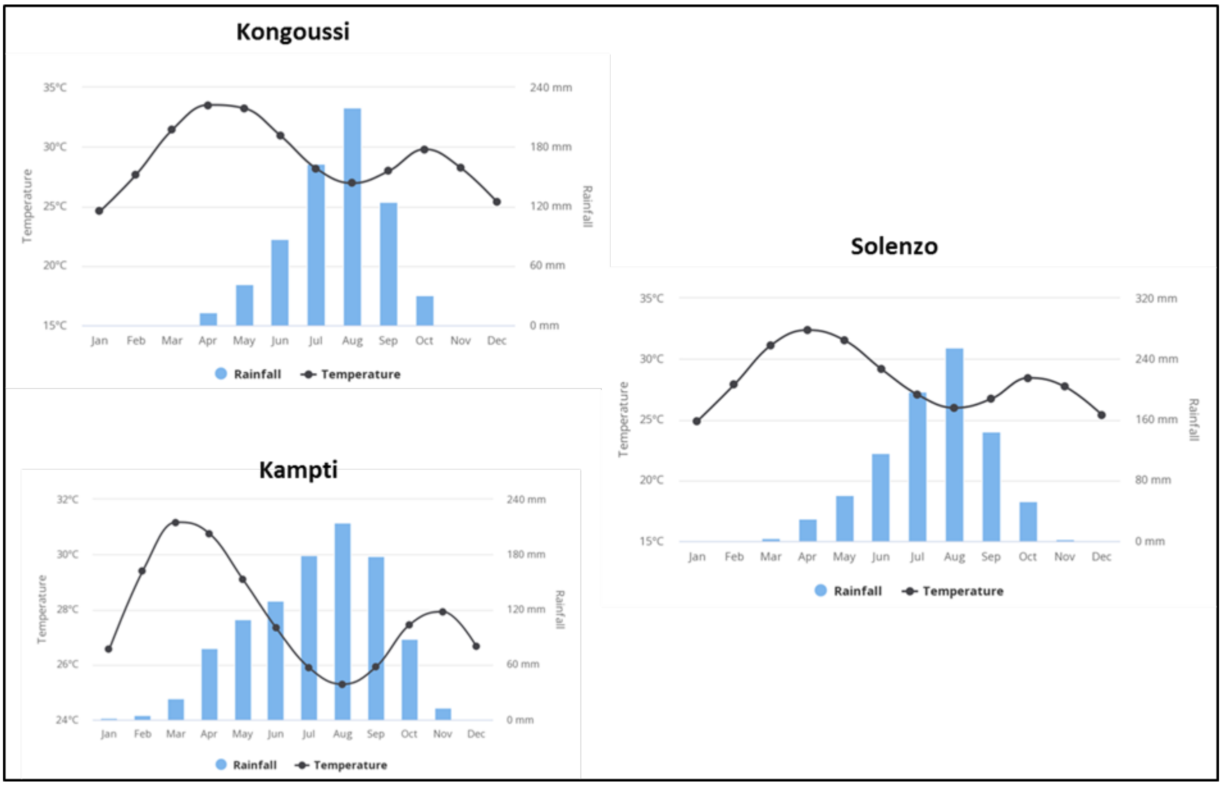


**Figure 2. Average monthly temperature and rainfall between 1991 and 2016 for the sprayed sites of Kongoussi, Solenzo and Kampti, used for longitudinal entomological monitoring by HLC and PSC.**




Mean climate data for the three IRS sites of Kongoussi, Solenzo and Kampti (Figure 2) shows the rainy season is short, lasting approximately four months from June to September in Kongoussi and Solenzo. In Kampti, the rainy season lasts longer due to the southerly location and lasts approximately six months, with rainfall between April and October. The mean temperature is high throughout the year in all sites, with mean temperature above 25°C for most of the year. Therefore, during the rainy season, conditions are ideal for *An. gambiae* s.l. proliferation.

## 2.2 Human Landing Catch (HLC)

Human landing catches (HLCs) were carried out in each site from 06:00 pm to 08:00 am in four randomly selected houses in the central sub-location and four houses in the rural sub-location (eight houses total per site per month, in the 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: one indoors (living room) and the second outdoors (within two meters of the house). The following morning, mosquito identification was performed using the key of Gillies and Coetzee (1987).

## 2.3 Pyrethrum Spray Catch (PSC)

Pyrethrum spray catches were conducted using 0.64 percent Pyrethrum EC aerosol insecticide. The houses were visited in the morning between 06:00 and 09:00 am and white sheets laid on the floor and over furniture. A total of 20 houses were selected per sub-location (central and rural) with a total of 40 houses surveyed per site per month. Ten to fifteen minutes after the spraying of houses, the knocked-down mosquitoes were collected from the white sheets. Mosquitoes were put in labelled petri dishes and were later morphologically identified. All female *Anopheles* were assessed for their abdominal status (unfed, fed, half gravid and gravid) and identified to species. Female *An. gambiae* s.l. were stored in 1.5 ml labelled Eppendorf tubes containing silical gel for further molecular analyses.

## 2.4 IRS quality Assurance and Residual Efficacy Monitoring (WHO Cone Tests)

The residual efficacy of insecticide on the walls was tested monthly by cone bioassays from June 2019 to February 2020 in IRS sites with WHO cone bioassays after spraying with Actellic® 300CS (Kongoussi Centre), SumiShield 50WG (Solenzo and Kongoussi rural sites), and Fludora Fusion WP-SB (Kampti). Wild *An. gambiae* s.l. larvae in each IRS site were collected and reared in the insectary to the adult stage, for monthly cone bioassay in parallel with the susceptible colony *An. gambiae* "Kisumu". A total of four treated houses (two houses made of cement and two houses made of mud) were randomly selected in each district for bioassay and monitored monthly until mortality was <80 percent.

The fumigant effect was tested by placing female *An. gambiae* s.l. in tubes at a distance of one meter from a treated wall. The team used a total of sixty unfed female *An. gambiae* Kisumu and *An. gambiae* s.l. from each site. They were exposed in four plastic tubes, with fifteen females tested per tube. Mosquito netting was placed at both ends to allow air to pass through. Females were exposed for 30 minutes and were then taken back to the insectary for delayed mortality assessment after 24 hours at 80 ± 10 percent relative humidity and 27 ± 2°C temperature. After exposure, mosquitoes were supplied with glucose solution and mortality was recorded 24 hours' post-exposure. The holding period was every 24h for up to seven days after exposure for houses sprayed with SumiShield 50WG and Fludora Fusion WP-SB to account for any delayed mortality.

## 2.5 Insecticide Susceptibility Tests

*An. gambiae* s.l. larvae were collected from different larval habitats from 12 localities, brought to the IRSS insectary and reared to adults prior to use in bioassays in order to assess the insecticide resistance status of adult *An. gambiae* s.l. The WHO tube tests were conducted to monitor insecticide susceptibility, pyrethroid resistance intensity and PBO synergist results using wild *An. gambiae* populations. Pyrethroid resistance intensity was monitored with alpha-cypermethrin, deltamethrin and permethrin at 5× and 10× the diagnostic concentration using the WHO tube protocol. The following insecticides were tested:

- Alpha-cypermethrin 0.05 percent, 0.25 percent, 0.50 percent
- Deltamethrin 0.05 percent, 0.25 percent, 0.50 percent
- Permethrin 0.75 percent, 3.75 percent, 7.50 percent
- Permethrin 0.75 percent + PBO 4 percent
- Deltamethrin 0.05 percent+ PBO 4 percent
- Pirimiphos-methyl 0.25 percent
- Clothianidin 2 percent
- Chlorfenapyr100µg/bottle

WHO criteria were used to classify the test populations as 'resistant' if less than 90 percent mortality was observed, moderate resistant if between 90-97 percent and susceptible if between 98-100 percent.

## 2.6 Laboratory Analyses

### 2.6.1 *Parity Rates of Anopheles Females*

Anophelines were morphologically identified to species using taxonomic keys of Gillies and De Meillon (1968) and Gillies and Coetzee (1987). A random sample of 50 unfed females per month collected from indoor and outdoor HLC were dissected, and the ovaries were observed to estimate the parity rate. Ovaries were dissected to determine parity rate, by observing the coiling of ovarian tracheoles (Detinova and Gillies, 1964). All specimens, including those dissected, were brought back to the IRSS laboratory and stored in the freezer at -20°C for further laboratory analyses.

### 2.6.2 *Plasmodium Falciparum Infection Rate*

All mosquitoes that were dissected for their parity status in the field were stored in a laboratory freezer at -20°C and subsequently processed by PCR to determine infectivity rates with *P. falciparum*. The head and thorax of all female *An. gambiae* s.l. specimens were used for PCR analyses as described by Morassin *et al.* (2002) and adapted by Sangaré *et al.* (2013). All samples that were tested for malaria infectivity rates were also identified to species level by PCR (Santalomazza et al., 2008).

### 2.6.3 *Mosquito Blood Meal Source*

Blood-fed females of *An. gambiae* s.l. from PSC were used to assess host preference for blood meal source. A random selection of specimens were tested by PCR using sequences of human, cow, pig, donkey and sheep blood (Kent & Norris, 2005). The same DNA-extracted process was used for mosquito species identification and blood-meal source PCR.

### 2.6.4 *Molecular Identification of An. gambiae Complex and Characterization of Resistance Mutations (kdr L1014/L1014S and ace-1R)*

A subsample of female *An. gambiae* s.l. were identified by PCR for species composition. Genomic DNA of mosquitoes was extracted with two percent cetyl trimethyl ammonium bromide (2 percent CTAB). Species of *An. gambiae* s.l. were identified and characterized, respectively by PCR Sine 200X 6.1 locus protocols of Santolamazza *et al.,* (2008). Detection of mutations involved in insecticide resistance was also performed by PCR using the protocol of Martinez-Torres *et al.,* (1998) and Ranson et al., (2000) for the *kdr* L1014F and L1014S mutations respectively and of Weill *et al.,* (2004) for the ace-*1*$^{R}$ G119S mutation.

## 2.7 Data Analysis

The human biting rate (HBR) was determined as the number of mosquitoes biting a person per night (indoor or outdoor). The *Anopheles* infection rate (IR) was calculated as the proportion of mosquitoes found to be positive for *P. falciparum* DNA in the head or thorax. The entomological inoculation rate (EIR) was defined as human biting rate multiplied by the *P. falciparum* infection rate and estimated as the number of infectious bites per human per month. A Chi-square test with the R statistical software (Version 3.4.0) was used to compare the mortality rates among the localities for susceptibility testing. An analysis of variance (ANOVA) was performed to compare the entomological estimates (HBR, IR) between sites. Data analysis was performed using R software, version 3.5.2. To analyse variables of interest (mosquito density, infectivity and entomological inoculation rates), the team fitted generalized linear mixed model (GLMM) using the glmmTMB function. In the case of the count variables, we used negative binomial families like nbinom2. A statistical difference was considered significant at p-value was less than 0.05.

# 3. Results

## 3.1 Malaria Vector Species Composition

Between June and December 2019, a total of 26,644 culicidae were collected by HLC including 14,152 anopheline mosquitoes. Other species including *Culex* (11,594), *Aedes* (685), *Mansonia* (213) and phlebotominae (641) were also collected by HLC indoors and outdoors (see Annex 1). Figure 3 presents overall morphological species data for all anophelines collected by HLC in eight longitudinal monitoring sites. A total of 12,957 culicidae were collected by PSC including 7,436 *An. gambiae* s.l. (60 percent), 4,944 *Culex* sp (37 percent) and 139 sandflies (see Annex 2).

*An. gambiae* s.l. was the most abundant by HLC, with 12,787 (53 %) followed by *An. nili* (38%) and a small number of *An. Funestus* (0.8%). The main malaria vectors collected in Burkina Faso were *An. gambiae* s.l., followed by small proportions of *An. nili* and *An. funestus* particularly. The abundance of *An. nili. An. funestus* and *An. coustani* was greater in Gaoua and Kampti in the Southwest from July to September, and very rare in Northern areas (Annex 1). *An. gambiae* sl was also the predominant species collected by PSC in all Anopheline species (7436/7857) (Figure 3B).

**Figure 3. *Anopheles* species composition based on A) HLCs and B) PSC for all sites combined.**

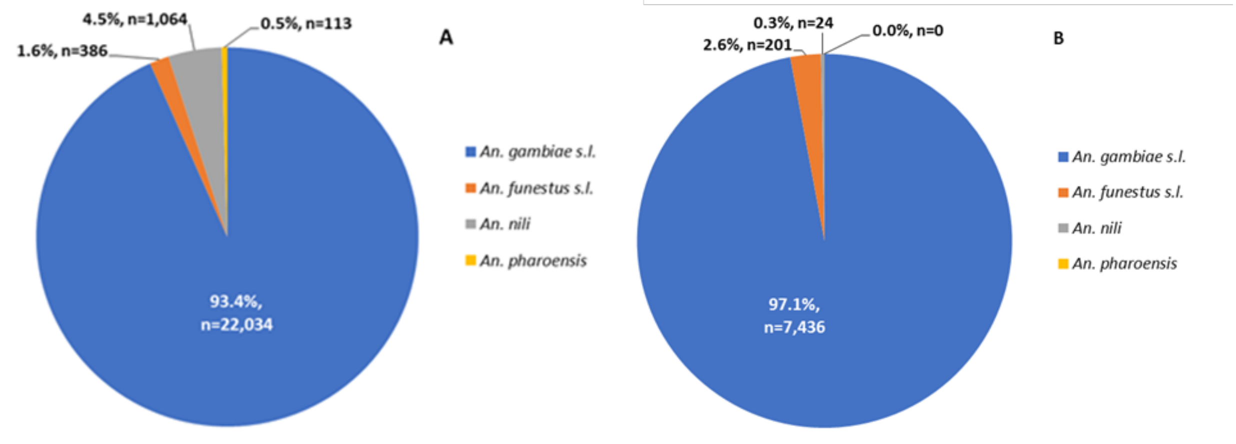
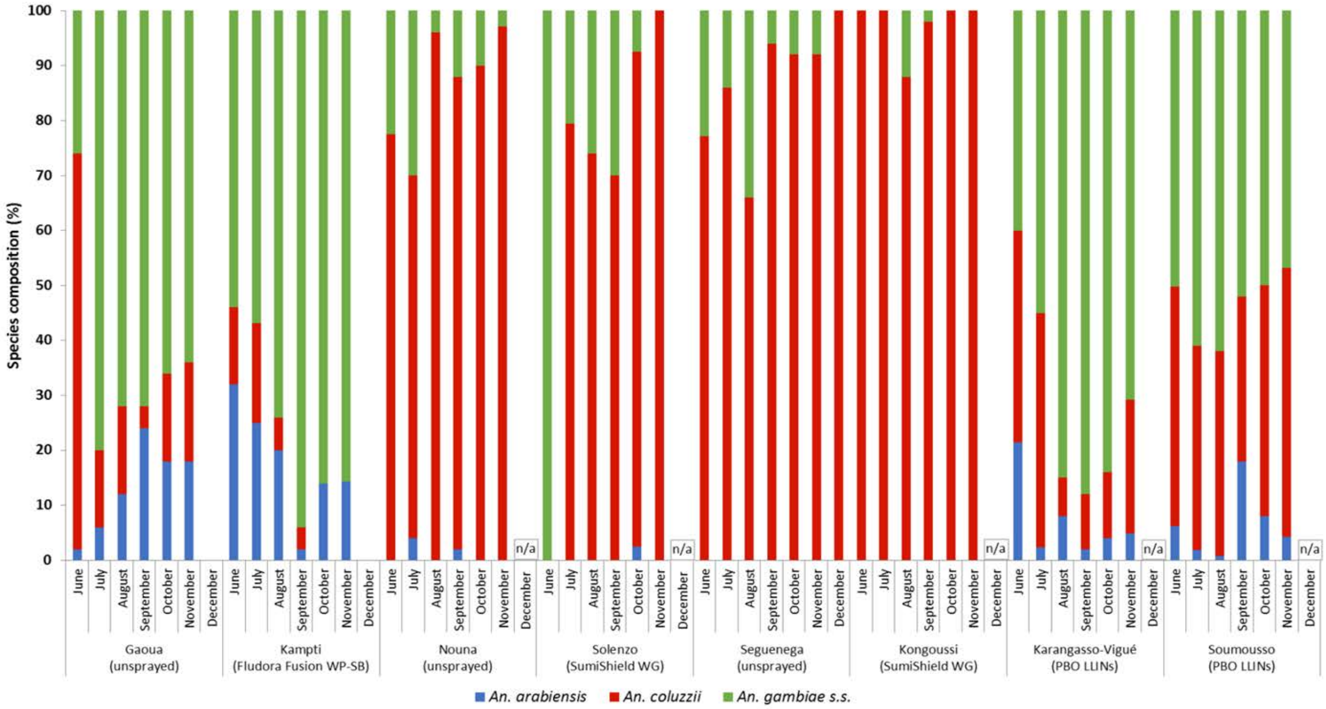



Figure 4 shows the members of the *An. gambiae* species complex identified by PCR by site from June to December. *An. coluzzii* was predominant in Nouna, Solenzo, Seguenega and Kongoussi. The majority of *Anopheles* in Kampti and Gaoua were *An. gambiae* during every month, except for June in Gaoua, where more *An. coluzzii* were collected. *An. arabiensis* were found every month at low proportions in Gaoua and Kampti, with the largest proportions of 30 percent. In Karangasso-Vigué and *An. gambiae* was also the predominant species for every month, although in Soumousso, there was close to a 1:1 ratio of *An. coluzzii* and *An. gambiae* throughout the year, which was observed in many previous studies (Dabiré *et al.*, 2007; Dabiré *et al.*, 2013). It is interesting that the proportion of *An. arabiensis* being collected in the Southwestern regions appears to be gradually increasing compared to previous year. In summary, *An.*

*gambiae* was the predominant malaria vector species in the Southwest (Gaoua, Kampti, Karangasso-Vigué and Soumousso), while *An. coluzzi* was more frequent in the North central (Seguenega and Kongoussi) and West (Nouna and Solenzo). In most sites, no mosquitoes were collected in December due to the dry conditions.

**Figure 4. Species composition within the *An. gambiae* complex from HLC in all sites (n=50 per month/site).**




## 3.2 *AN. GAMBIAE* S.L. HUMAN BITING RATE (HBR)

Figure 5 presents the mean monthly indoor and outdoor *An. gambiae* s.l. human biting dynamics in all IRS and the unsprayed control sites (combined data for 1 rural and 1 central location for each site). At the end of the dry season in June, when IRS application took place, the biting rates were generally low in all sites except in Gaoua and Kampti with mean indoors and outdoors biting rates of >5 bites/person/night (b/p/n). The number of bites increased monthly after June, with peak biting rates reached in August and September (indoors and outdoors) for all sites. Comparison of unsprayed sites with their paired sprayed sites showed that mean indoor and outdoor biting rates were consistently lower in Kongoussi (Actellic CS/SumiShield WG) than in Seguenega (unsprayed); however, biting rates were far greater in Solenzo (SumiShield WG) than in Nouna (unsprayed). There was no clear difference in biting rates for Kampti (Fludora Fusion WP-SB) and Gaoua (unsprayed).

While the impact of IRS on biting rates appeared to be minimal when the data was broken down to rural and central sub-locations, there were consistently lower biting rates from July to December in the spayed semi-urban central sites compared to paired control sites (Annex 1). However, in rural sites, the biting rates were generally far higher than central sites and there was no apparent impact of IRS on biting rates.

PBO-synergist ITNs were distributed between June 28 and July 3, 2019 in Karangasso-Vigué and Soumousso. From July to December in both sites, the biting frequency increased monthly until a peak was reached in September/October at 10-13 bites per person per night indoors and 12-19 outdoors (Figure 6).

**Figure 5. Mean *An. gambiae* s.l. bites per person per night from indoor (A) and outdoor (B) HLC collections from June to December 2019.**

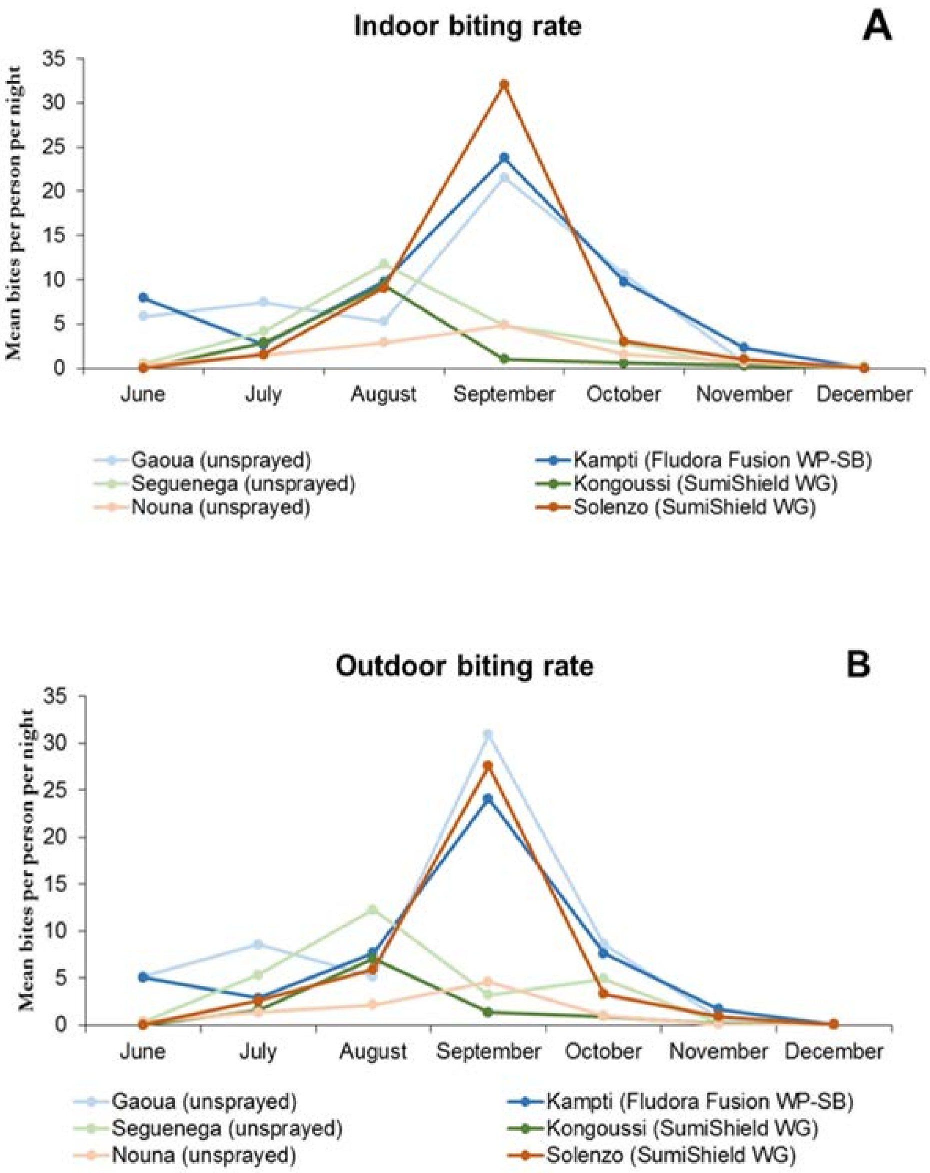
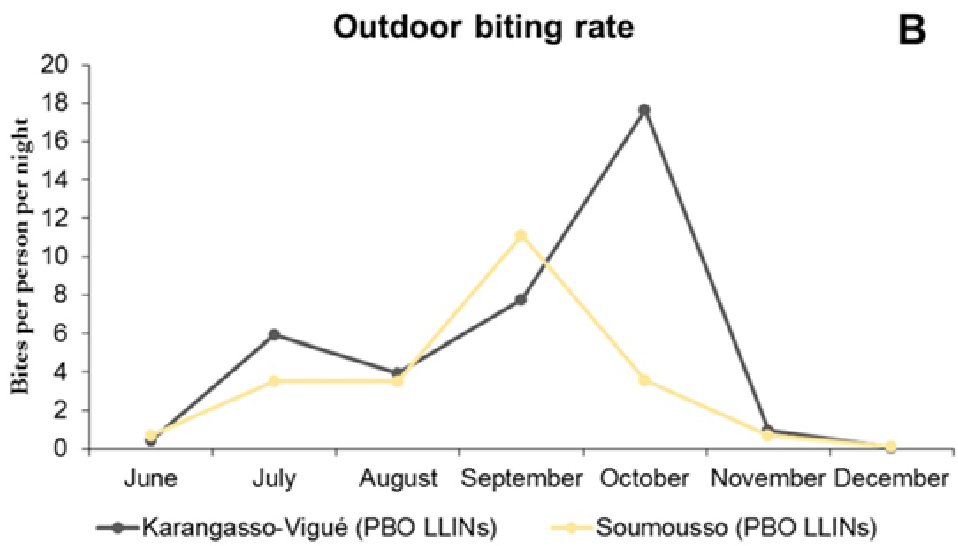
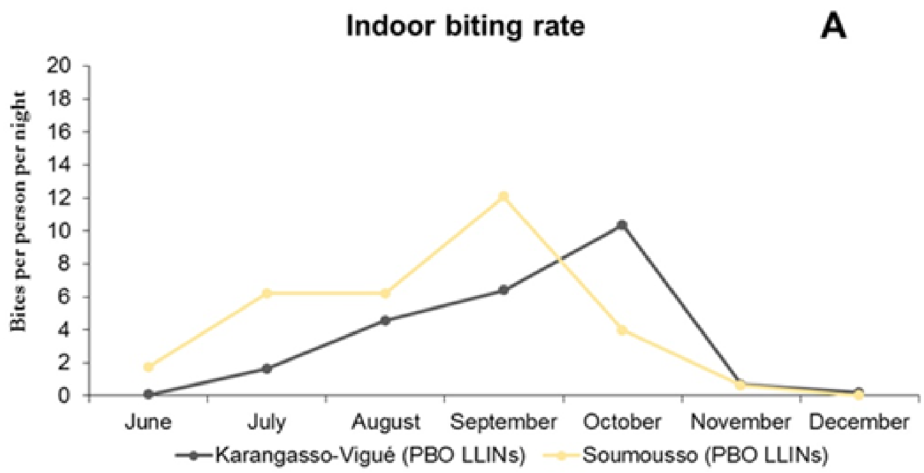


**Figure 6. Mean *An. gambiae* s.l. bites per person per night indoors (A) and outdoors (B) from June to December 2019 in HLC collections from PBO LLINs sites.**




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

Figures 7-8 show the mean hourly indoor and outdoor biting times of *An. gambiae* s.l. in IRS and unsprayed sites, while figure 9 shows biting times in PBO LLIN sites. The trends were broadly similar in all sites, with classic late night biting resulting in peak indoor and outdoor biting between 11pm and 5am. In Kongoussi and Seguenega, the peak biting times were slightly earlier, with the peak between 9pm and 3am.

**Figure 7. Biting times of *An. gambiae* s.l. per hour from indoor HLC collections from June to December 2019.**

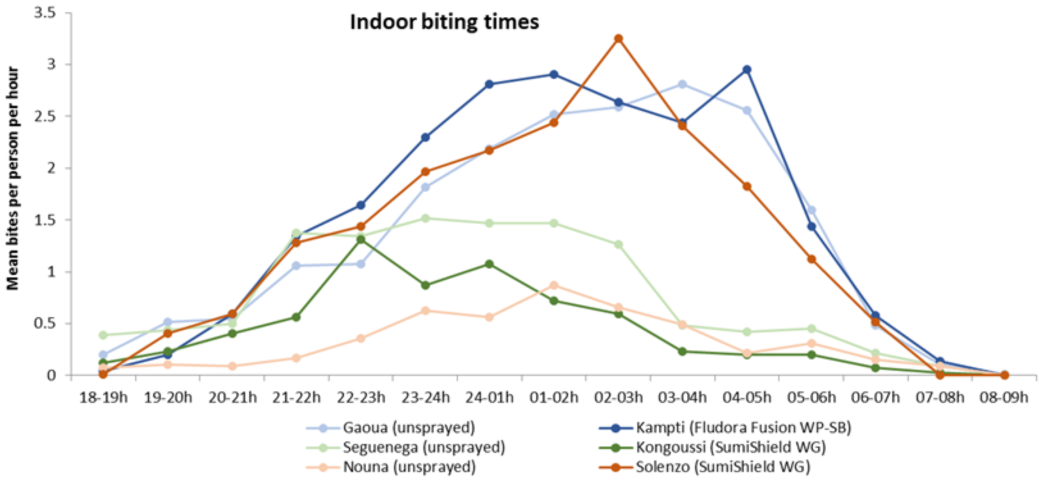



**Figure 8. Biting times of *An. gambiae* s.l. per hour from outdoor HLC collections from June to December 2019.**

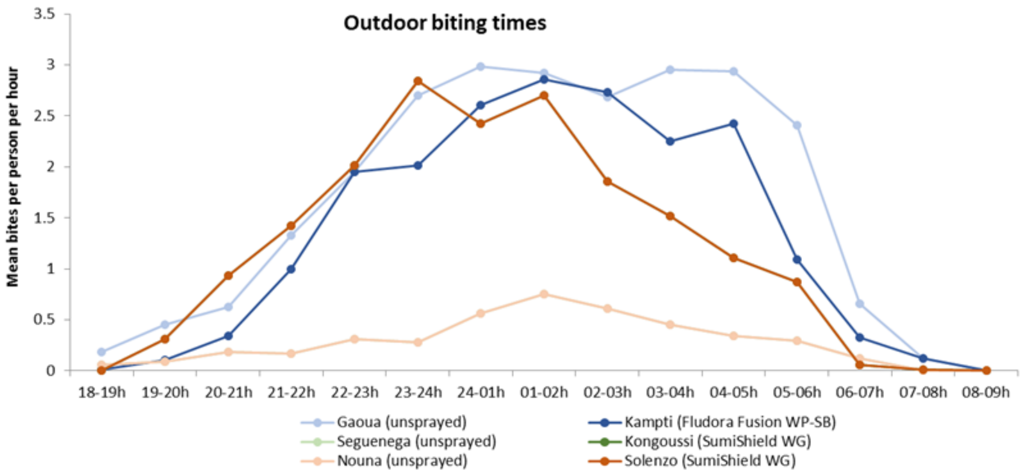
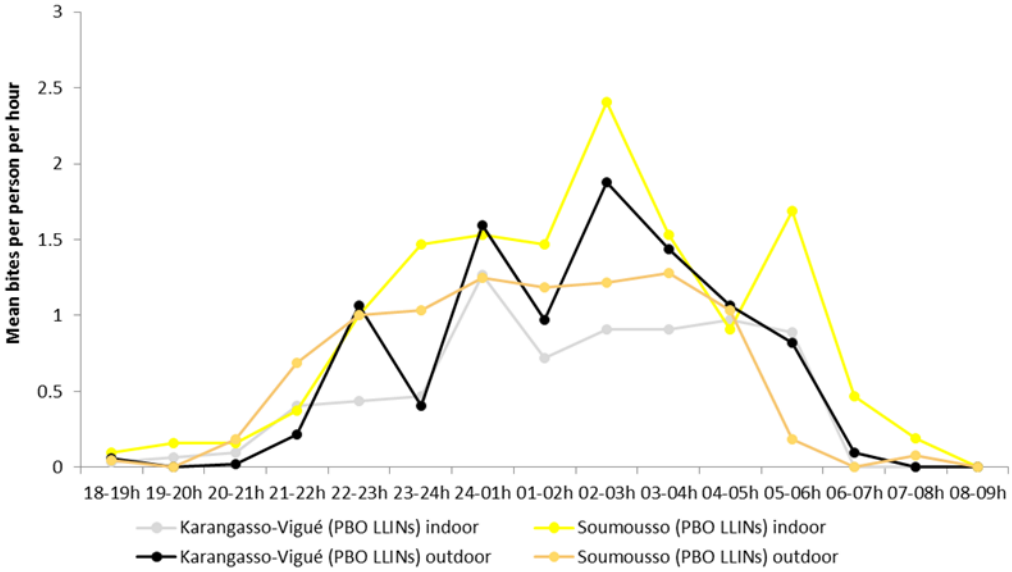


**Figure 9. Biting times of *An. gambiae* s.l. per hour from indoor and outdoor HLC collections from June to December 2019 in PBO LLINs sites.**

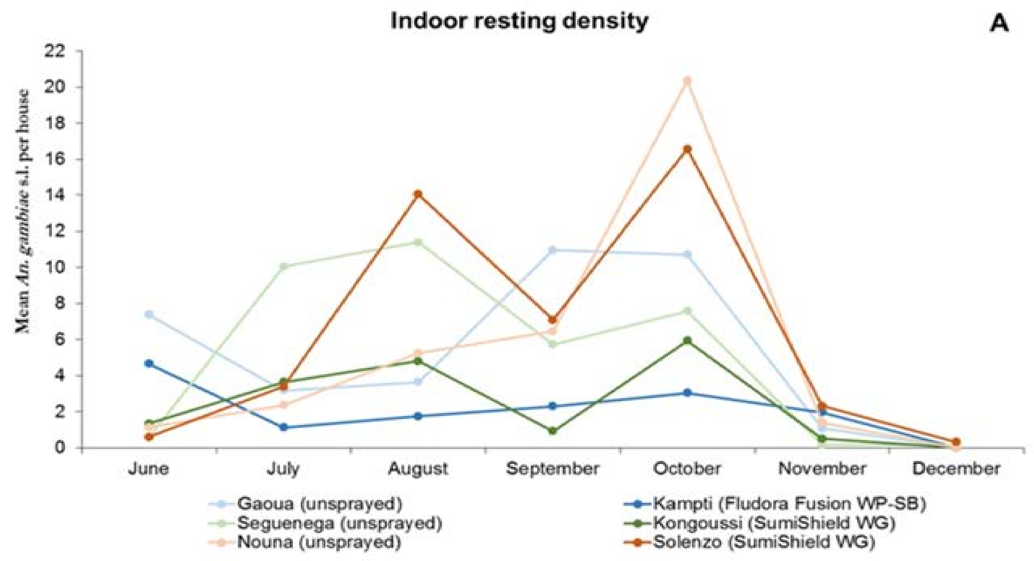


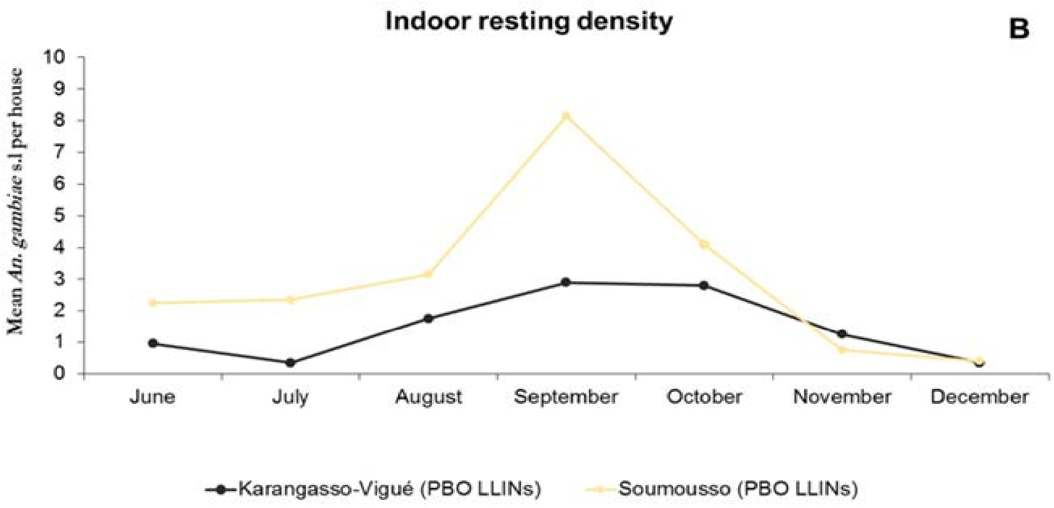

## 3.4 Indoor Resting Densities by PSC

The density of *An. gambiae* s.l. collected from PSC was generally lower in IRS sites than their paired unsprayed control sites (Figure 10), particularly in Kongoussi and Kampti where densities were < 6 *An. gambiae* s.l. per house per day. In Kampti (sprayed with Fludora Fusion WP-SB), the density was < 3 *An. gambiae* s.l. per house/night from July to December which was statistically lower compared to Gaoua (RR= 3.97 [2.65, 5.95], Tukey's p< 0.001). However, in the sprayed site of Solenzo (SumiShield WG), the densities were not significantly different from the unsprayed site of Nouna with a mean of 17 *An. gambiae* s.l. per day in October, four months after IRS application.

The impact of IRS appeared to be different in central and rural sub-locations. Annex 2 shows that densities from PSC collections were always considerably lower in sprayed central sites compared to unsprayed central sites. However, in rural sites where densities were generally far greater, there was no clear impact of IRS on indoor resting densities.

**Figure 10. Mean *An. gambiae* s.l. per house per month from indoor PSC in (A) IRS and unsprayed sites and (B) PBO LLIN sites.**




## 3.5 Parity Rate of *An. gambiae* s.l.

Table 1 summarizes the overall parity rate from monthly dissections between June and December 2019 by site. Comparison of sprayed sites with paired unsprayed sites shows that the parity rate in the sprayed district of Kongoussi (63 percent) was lower than in the unsprayed district of Seguenega (78 percent) ($\chi^2 = 16.01$, df = 1, $p<0.0001$) and lower in Solenzo at 70 percent (SumiShield WG) than in Nouna (unsprayed) at 78 percent. There was no apparent difference in parity rate between Gaoua and Kampti sites.

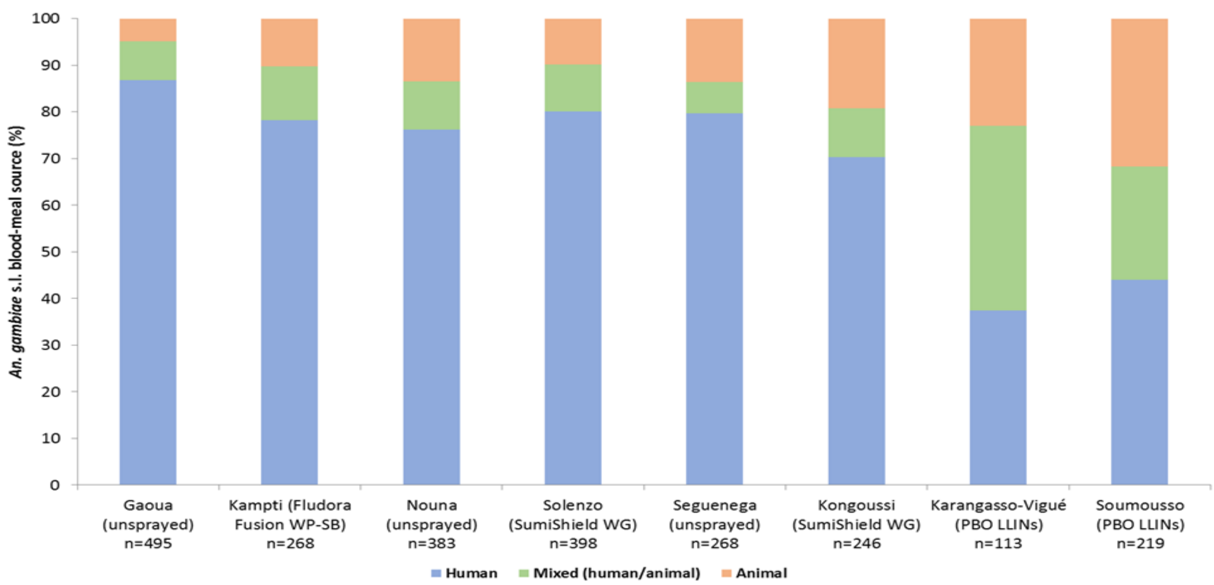
**Table 1. Parity rate of An. gambiae s.l. females collected between June and December 2019.**

| Site | Total dissected | Parous | Non-parous | Parity rate (%) |
|---|---|---|---|---|
| Gaoua (unsprayed) | 910 | 674 | 236 | 74.1 |
| Kampti (Fludora Fusion WP-SB) | 818 | 628 | 190 | 76.8 |
| Nouna (unsprayed) | 330 | 257 | 73 | 77.9 |
| Solenzo (Sumishield WG) | 554 | 387 | 167 | 69.9 |
| Seguenega (unsprayed) | 412 | 323 | 89 | 78.4 |
| Kongoussi (Actellic CS/SumiShield WG) | 327 | 206 | 121 | 63.0 |
| Karangasso-Vigué (PBO LLINs) | 309 | 195 | 114 | 63.1 |
| Soumousso (PBO LLINs) | 292 | 195 | 97 | 66.8 |

## 3.6 *AN. GAMBIAE* S.L. BLOOD-MEAL SOURCE

*An. gambiae* s.l. were extremely anthropophilic in all sites without any difference between sprayed and control sites (Figure 11). The proportion of strictly zoophagic *An. gambiae* was very low, with a maximum of 10 percent in sprayed (Seguenega) and unsprayed sites and 30 percent in the PBO LLIN sites. Mixed human and animal blood meals were relatively common and accounted for 10 percent of samples in most sites and up to 45 percent in Karangasso-Vigué. The majority of mixed blood-meals were a combination of human and cattle blood. In some sites, several households used cattle for field ploughing, which may explain the mixed or animal blood feeding patterns observed. The proportion of blood-meals that contained human blood (including mixed blood-meals) was >80 percent in all sites except the sites with PBO nets. More evidence is needed before any conclusions can be made about whether PBO nets have altered *An. gambiae* s.l. feeding behavior.

**Figure 11. Blood-meal source of *An. gambiae* s.l. collected by PSC between June and December 2019.**



## 3.7 *P. falciparum* Infection Rates of *Anopheles gambiae* s.l.

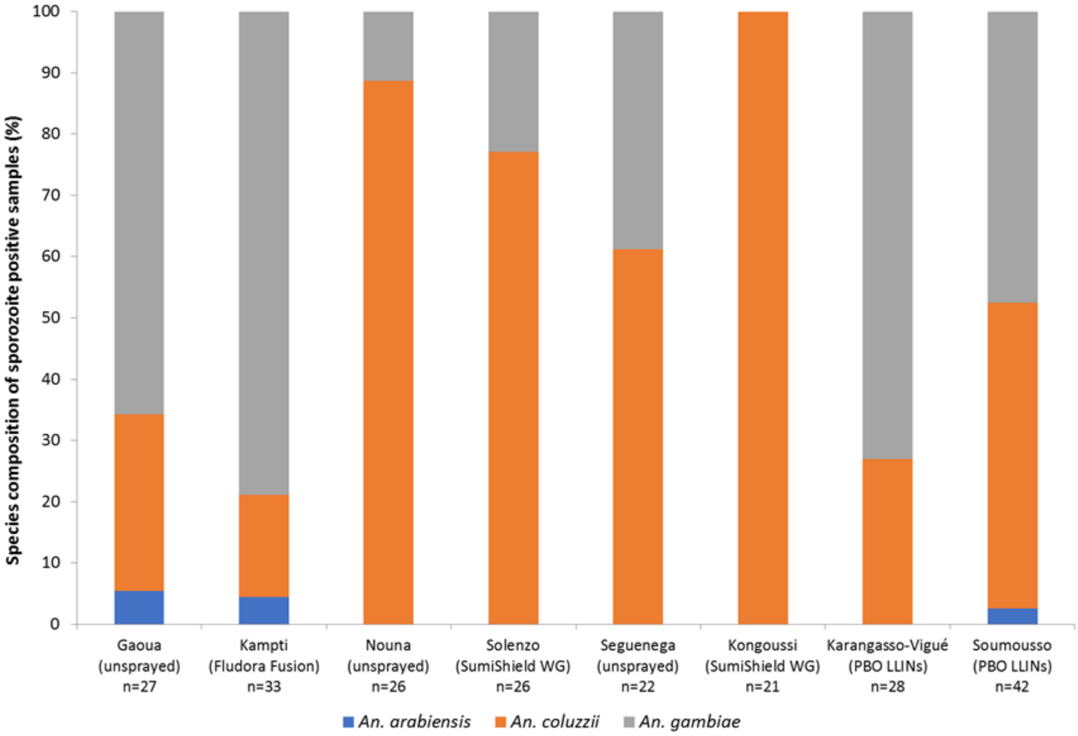
*P. falciparum* infection rates in *An. gambiae* s.l. were high in all sites, regardless of vector control intervention (Table 2). In the rural sites, the infection rates were higher than in central sites with some values reaching 20 percent in Gaoua and Kampti (Annex 4). The mean infection rates did not differ between sprayed and unsprayed sites.

**Table 2. *An. gambiae* s.l. sporozoite rate (*P. falciparum*) per site from June to December 2019.**

| Site | Total *An. gambiae* s.l. tested/total collected by HLC) | % Sporozoite rate (positive/ tested) |
|---|---|---|
| Gaoua (unsprayed) | 300/1,863 | 9.00% (27/300) |
| Kampti (Fludora Fusion WP-SB) | 251/886 | 13.14% (33/251) |
| Nouna (unsprayed) | 275/467 | 9.45% (26/275) |
| Solenzo (SumiShield WG) | 185/307 | 14.05% (26/185) |
| Seguenega (unsprayed) | 258/1,533 | 8.52% (22/258) |
| Kongoussi (SumiShield WG) | 186/608 | 11.29% (21/186) |
| Karangasso-Vigué (PBO LLINs) | 254/966 | 11.02% (28/254 |
| Soumousso (PBO LLINs) | 297/843 | 14.14% (42/297 |

*Anopheles* species composition was determined for those samples that were positive for *P. falciparum* sporozoites (Figure 12). The composition was similar to those presented in Figure 4. *An. gambiae* was the most common malaria vector in the Southwest (Gaoua, Kampti, Karangasso-Vigué and Soumousso). *An. coluzzii* was the main vector in Solenzo, Nouna, Kongoussi and Seguenega. Infected *An. arabiensis* were found in Gaoua, Kampti and Soumousso but in low numbers. The sporozoite rates in Burkina Faso are extremely high and there is the possibility that this could be an overestimate if PCR is detecting *P. falciparum* DNA from pre-sporozoite stages. The 2019 sporozoite rates in neighboring Mopti Region of Mali were far lower with < 2% by ELISA assays. Since sporozoite rate is a critical factor for EIR, it would be useful to compare PCR and ELISA assay for the estimation of sporozoite rates and validate the EIRs results in Burkina Faso.

**Figure 12. Species composition of P. falciparum infected female An. gambiae s.l. from June to December 2019.**




## 3.8 ENTOMOLOGICAL INOCULATION RATE

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 (June to December = 214 days). Results are presented in table 3 using sporozoite rates calculated from *An. gambiae s.l.* collected by indoor and outdoor HLC. The EIR was high in the Southwest, with Gaoua (unsprayed) having an EIR of 160 infective bites per person between June and December 2019. The EIR was lower in neighboring Kampti (Fludora Fusion), but the malaria transmission risk was still extremely high with 109 infective bites per person. The EIR was similar in Nouna (unsprayed) with 42 infective bites per person compared with 41 in Solenzo (SumiShield WG). In central sites of Kongoussi, the EIR (66 infective bites per person) was significantly lower than Seguenega (125 infective bites per person). The overall EIRs (indoor and outdoor) was two-fold lower in Kongoussi compared to Seguenega (RR=4.73, IC95 percent= [1.41–15.87], p=0.011). In the two sites where PBO LLINs were distributed, the EIR was high with 102 infectious bites per person in Karangasso-Vigué and 114 in Soumousso. These results highlight that despite IRS and PBO LLIN distribution, EIR remains high in all sites, largely due to the high sporozoite rates recorded.

**Table 3. An. *gambiae* s.l. entomological inoculation rate (June to December 2019)**

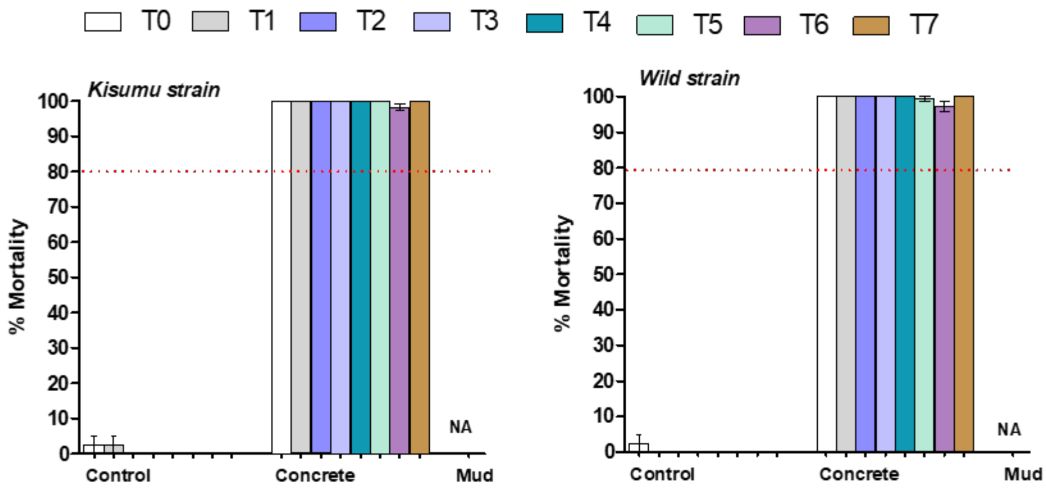
| | Gaoua (unsprayed) | Kampti (Fludora Fusion WP-SB) | Nouna (unsprayed) | Solenzo (SumiShield WG) | Seguenega (unsprayed) | Kongoussi (Actellic CS/ SumiShield WG) | Karangasso-Vigué (PBO LLINs) | Soumousso (PBO LLINs) |
|---|---|---|---|---|---|---|---|---|
| Total *An. gambiae* s.l. collected (HLC) | 1,863 | 886 | 467 | 307 | 1,533 | 608 | 966 | 843 |
| HLC trap-nights (indoors + outdoors) | 224 | 224 | 224 | 224 | 224 | 224 | 224 | 224 |
| HBR per night | 8.31 | 3.96 | 2.08 | 1.37 | 6.84 | 2.71 | 4.31 | 3.76 |
| Total *An. gambiae* s.l. tested by PCR (HLC) | 565 | 526 | 407 | 439 | 403 | 399 | 254 | 297 |
| Sporozoite rate | 9.00% | 13.14% | 9.45% | 14.05% | 8.52% | 11.29% | 11.02 | 14.14 |
| EIR per night | 0.749 | 0.520 | 0.197 | 0.192 | 0.584 | 0.306 | 0.475 | 0.532 |
| **EIR June to December 2019 (214 nights)** (calculated using mean sporozoite rate) | **160** | **111** | **42** | **41** | **125** | **66** | **102** | **114** |

## 3.9 Residual Efficacy of SumiShield 50WG, Fludora Fusion and Actellic 300CS against Susceptible *An. gambiae* "Kisumu" and Wild *An. gambiae* s.l.

Each month from June to December, the residual efficacy of each treatment (Actellic 300CS, SumiShield 50WG, and Fludora Fusion WP-SB), in terms of mortality, was evaluated by WHO cone tests using the susceptible "Kisumu" and wild *An. gambiae* s.l. mosquitoes from each locality namely Kongoussi, Solenzo and Kampti. In Kongoussi center, sprayed with Actellic® the mortality rate of Kisumu strains was 100 percent *for* the first five months and between 98-100 percent over the seven months (Figure 13). The tests performed with wild strain of *An. gambiae* indicated also high mortality rates of 100 percent. The mortality rates of control bioassays on unsprayed walls was less than 5 percent.

**Figure 13. Mortality rate (24h) of An. gambiae Kisumu and wild An. gambiae s.l. using monthly WHO cone bioassay on walls sprayed with Actellic 300CS in Kongoussi.**

**Red dashed line represents the WHO threshold of 80 percent mortality. T0 to T7: = 0 to 7 months after** spraying.




In Solenzo where houses were sprayed with SumiShield 50WG, the mortality rates reached 100 percent over a period of seven months with walls made with mud and cement both for Kisumu and wild *An. gambiae* s.l. (Figure14).

**Figure 14. Mortality rates (120h) of *An. gambiae* Kisumu and wild *An. gambiae* s.l. using monthly WHO cone bioassay on walls sprayed with SumiShield 50WG in Solenzo.**

Error bars represent the 95 percent confidence interval. Red dashed line represents the WHO threshold of 80 percent mortality. T0 to T7: = 0 to 7 months after spraying

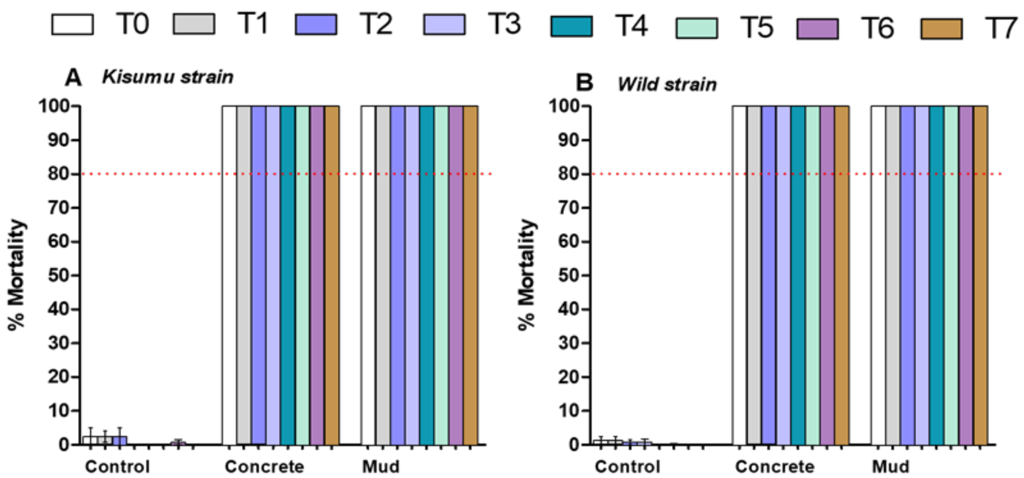
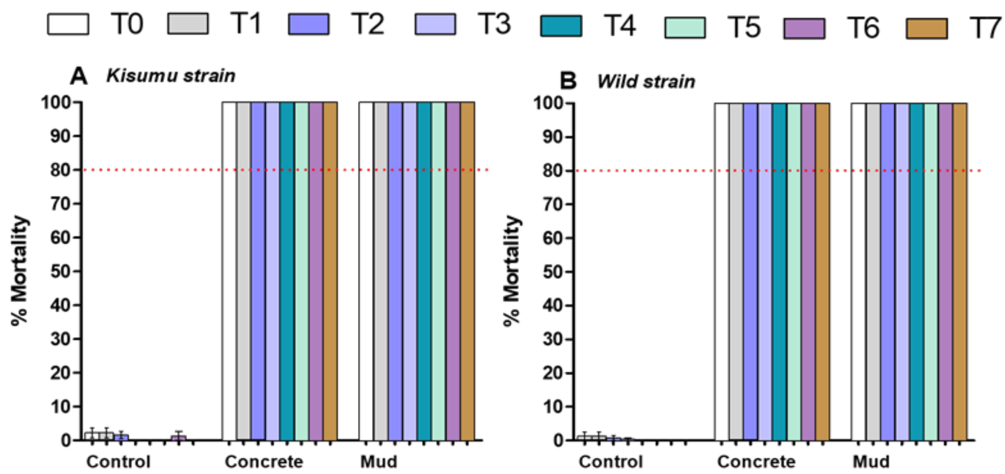


**Figure 15. Mortality rate (120h) of *An. gambiae* Kisumu and wild *An. gambiae* s.l. using monthly WHO cone bioassay on walls sprayed with Fludora Fusion WP-SB in Kampti.**

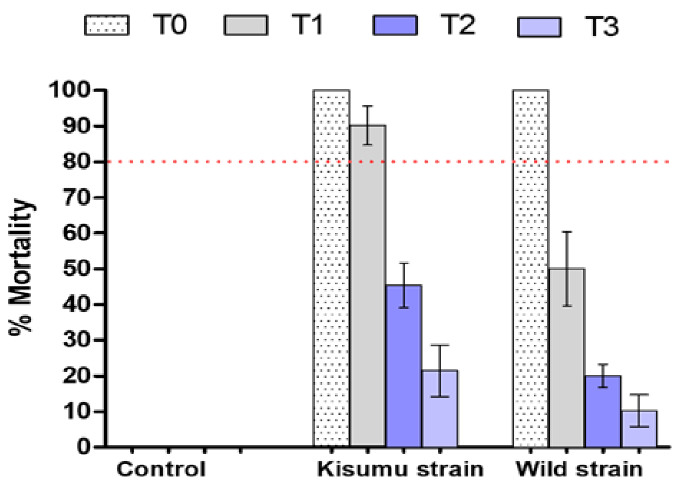
Error bars represent the 95 percent confidence interval. Red dashed line represents the WHO threshold of 80 percent mortality. T0 to T7: = 0 to 7 months after spraying.




In Kampti, mortality of 100 percent was observed both on cement and mud walls with *An. gambiae* "Kisumu" and also with wild *An. gambiae* s.l. (Figure 15). Results of cone bioassay show that all insecticide formulations sprayed were effective during seven months of monitoring. Mortality will continue to be monitored until it is <80 percent for two consecutive months.

**Figure 16. Fumigant assay (24h mortality) of *An. gambiae* Kisumu and wild *An. gambiae* s.l. in houses sprayed with Actellic CS in Kongoussi.**

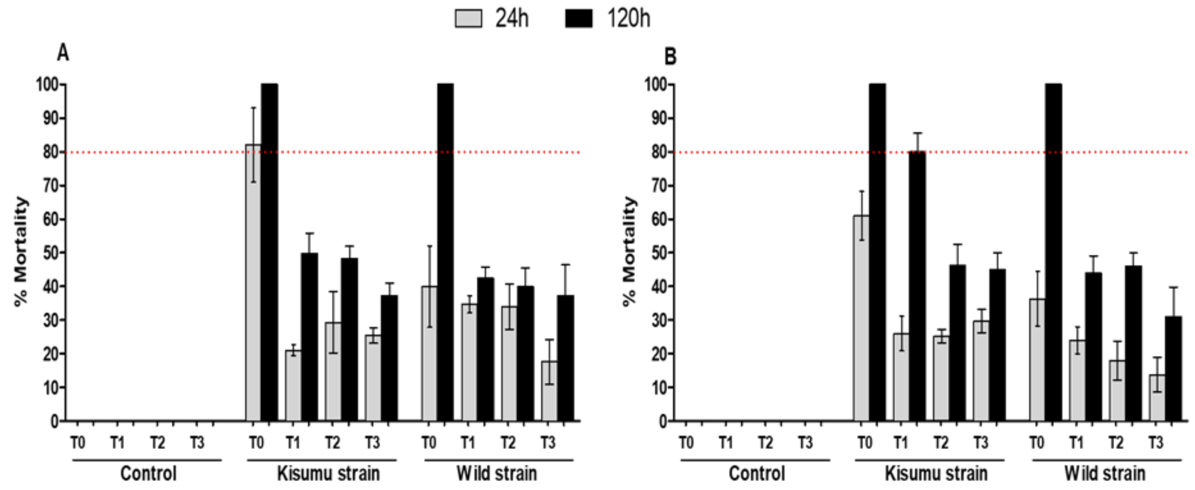
Error bars represent the 95 percent confidence interval. Red dashed line represents the WHO threshold of 80 percent mortality. T0 to T3: = 0 to 3 months after spraying.




The airborne fumigant effect produced a mortality rate of 100 percent both for susceptible *An. gambiae* Kisumu and wild *An. gambiae* s.l. in Kongoussi center at T0, reaching 90 percent at T1 with Kisumu and 50 percent with the wild *An. gambiae* s.l. The fumigant effect was short-lived, with mortality <30 percent after three months (Figure 16).

**Figure 17. Fumigant assay (24h and 120h mortality) of *An. gambiae* Kisumu and wild *An. gambiae* s.l. in Solenzo district sprayed with SumiShield WG.**

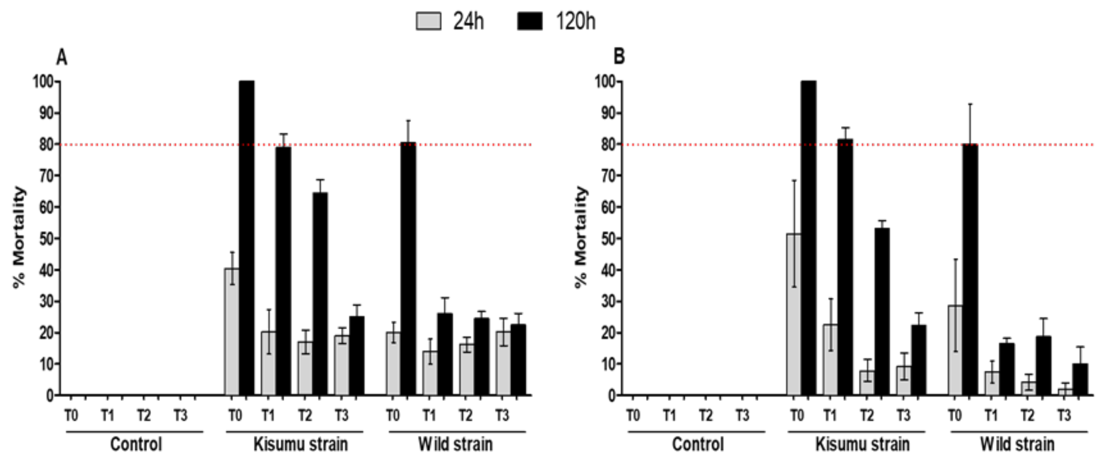
Error bars represent the 95 percent confidence interval. Red dashed line represents the WHO threshold of 80 percent mortality. T0 to T3: = 0 to 3 months after spraying.




In Solenzo center and rural village of Mole, the airborne fumigant effect was highly effective shortly after spraying, with a mortality rate of 100 percent both for *An. gambiae* Kisumu and wild *An. gambiae* s.l. after a 120-hour observation. There was a reduction in mortality just one month after spraying of 50-80%, and after three months, mortality was around 40 percent for both *An. gambiae* Kisumu and wild *An. gambiae* s.l. (Figure 17).

**Figure 18. Fumigant assay (24h and 120h mortality) of *An. gambiae* Kisumu and wild *An. gambiae* s.l. in Kampti district sprayed with Fludora Fusion WP-SB.**

Error bars represent the 95 percent confidence interval. Red dashed line represents the WHO threshold of 80 percent mortality. T0 to T3: = 0 to 3 months after spraying.

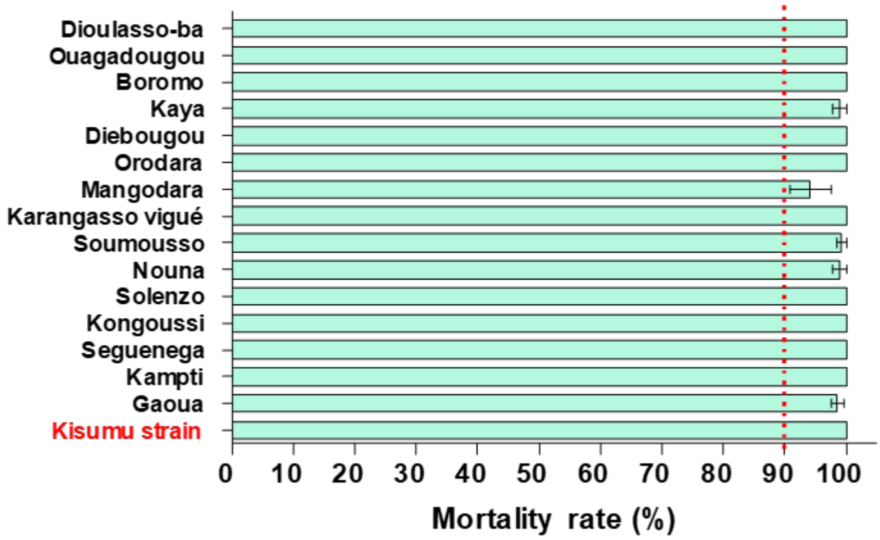



The fumigant effect of Fludora Fusion WP-SB was relatively high for two months after spraying, but was short-lived and reduced to <30 percent by three months after spraying.

## 3.10 INSECTICIDE SUSCEPTIBILITY DATA

Figure 19 summarizes the results of susceptibility tests performed with wild *An. gambiae* s.l. that were collected as larvae and reared to adults before being tested with pirimiphos-methyl (PM) 0.25 percent. Susceptibility (98-100 percent) was recorded 24 hours after exposure in 14 of 15 sites, including the three IRS sites (Figure 19). There was possible resistance (90-98 percent mortality) recorded in Mangodara (non-IRS site).

**Figure 19. Results of susceptibility tests with 0.25 percent pirimiphos-methyl in WHO tubes against wild *An. gambiae* s.l. reared from larval collection from 15 sites.**




*An. gambiae* s.l. were resistant in all 15 sites to both deltamethrin 0.05 percent and permethrin 0.75 percent. When pre-exposed to PBO, followed by deltamethrin, the mortality rate increased significantly in all 15 sites, reaching up to 90-98 percent in seven sites (Figure 20A). Similar trends were observed with PBO + permethrin and there was an increase in mortality in all sites compared with permethrin alone. However, the increase in mortality was limited and mortality was >90 percent in only three sites after PBO pre-exposure followed by permethrin (Figure 20B). Of note, results for Karangasso-Vigué and Soumousso, where PermaNet 3.0 LLINs (containing deltamethrin + PBO) were distributed in June 2019. Mortality with PBO + deltamethrin was 95 percent in Karangasso-Vigué, from 50 percent with deltamethrin alone and 97 percent in Soumousso, from 20 percent with deltamethrin alone. These results are extremely encouraging and indicate that PermaNet 3.0 should provide greater control in these sites than pyrethroid only LLINs. In conclusion, pre-exposure to PBO followed by deltamethrin or permethrin resulted in substantial increases in mortality, reaching >80 percent in most sites indicating that metabolic mechanisms play an important role in phenotypic resistance.

**Figure 20. Results of synergist tests with (A) deltamethrin 0.05 percent and PBO 4 percent + deltamethrin 0.05 percent and (B) permethrin 0.75 percent and PBO 4 percent + permethrin 0.75 percent**

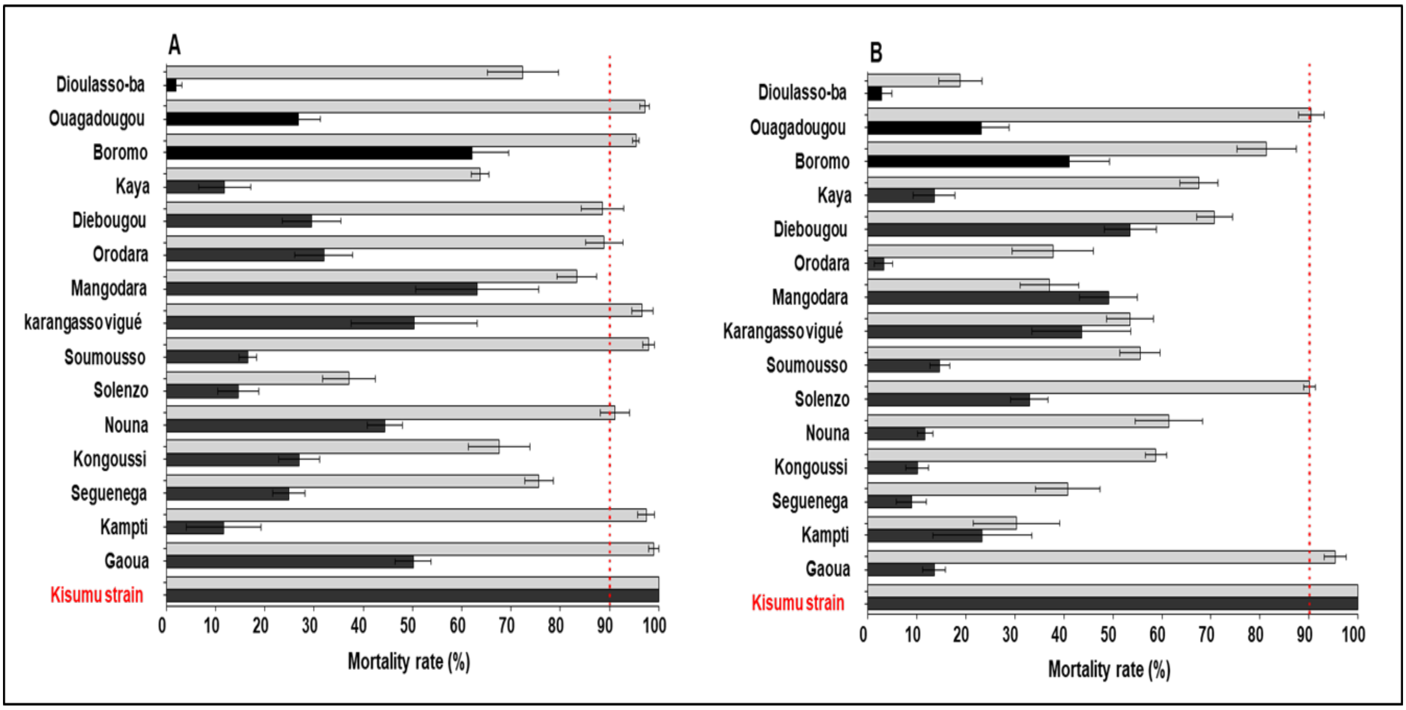



Figure 21 shows the results of susceptibility tests performed on *An. gambiae* s.l. against chlorfenapyr 100µg/bottle. There was high mortality (98-100 percent) just 24 hours after exposure in most sites, except in Gaoua, Kongoussi and Solenzo where <80 percent mortality was observed. However, after 72 hours, tests in all sites reached 98 to 100 percent mortality, except in Boromo where it remained <95 percent. These results indicate that wild *An. gambiae* s.l. from these sites are susceptible to chlorfenapyr in Burkina Faso.

Mortality rates obtained 24 hours after exposure with clothianidin 2 percent varied between 90 percent in Kampti (the lowest) and 100 percent (the highest) in Kongoussi, Seguenega and Karangasso Vigué (Figure 22). After 120 hours, all sites reached 100 percent mortality, including in all three IRS sites (Figure 22). Therefore, SumiShield WG and Fludora Fusion WP-SB can continue to be used for IRS as part of a rotation strategy.

**Figure 21. Results of susceptibility tests with chlorfenapyr 100µg/bottle in CDC bottle bioassays against *An. gambiae* s.l. in 13 sites.**

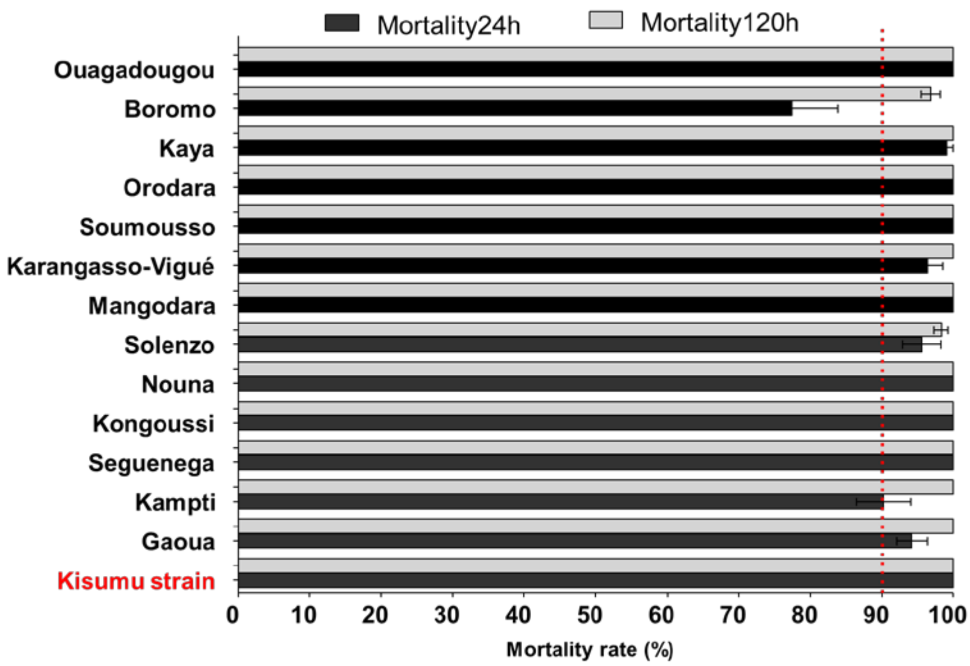
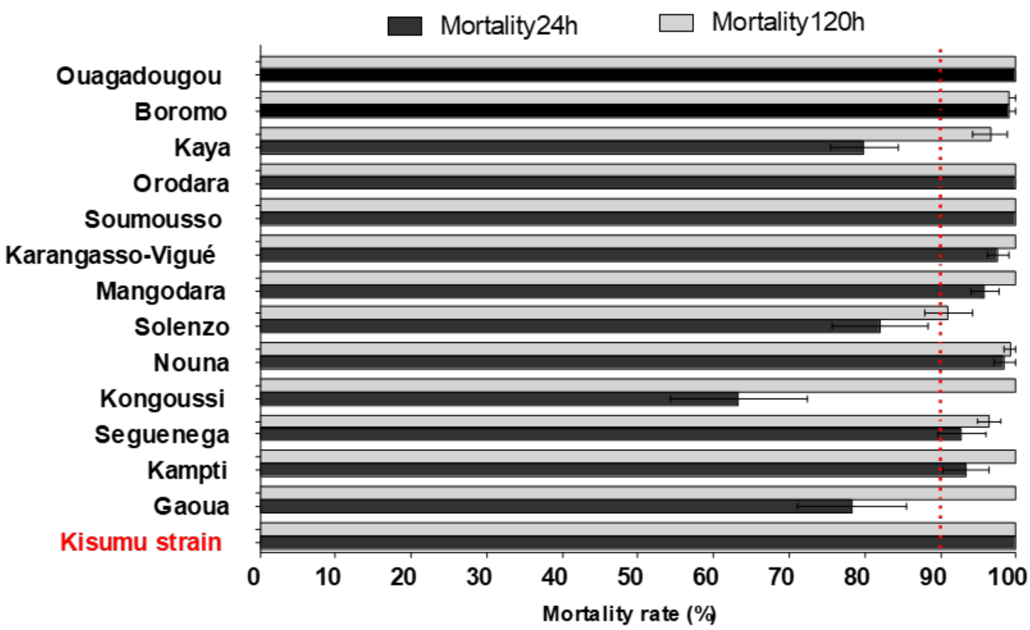



**Figure 22. Results of susceptibility tests with clothianidin 2 percent papers in WHO tube tests against *An. gambiae* s.l. in 13 sites.**



## 3.11 Resistance intensity (WHO tube bioassay)

The results of resistance intensity tests for *An. gambiae* s.l. exposed in WHO test tubes to alpha cypermethrin and deltamethrin 5x and 10x showed high resistance intensity in all sites (Tables 4 and 5). The results were more variable for permethrin, with high intensity resistance in six sites, moderate intensity in three sites and low intensity in one site (Table 6).

**Table 4. Percentage mortality of *An. gambiae* s.l. after exposure to deltamethrin at ×1, ×5, and ×10 the diagnostic concentration in WHO tube tests in 14 sites.**

| Sites | Deltamethrin diagnostic concentration | | | | | | Status |
|---|---|---|---|---|---|---|---|
| | 1x | | 5x | | 10x | | |
| | N | % mortality [CI] | N | % mortality [CI] | n | % mortality [CI] | |
| Kisumu strain | 100 | 100 | 100 | 100 | NA | NA | Susceptible colony |
| Gaoua | 102 | 50.09 [38.57-61.61] | 107 | 86.94 [80.03-93.84] | 115 | 93.05 [88.43-97.66] | High resistance intensity |
| Kampti | 88 | 11.63 [00.00-44.15] | 121 | 40.13 [25.33-54.92] | 112 | 71.47 [68.60-74.34] | |
| Kongoussi | 106 | 26.97 [13.89-40.04] | 100 | 59.55 [39.11-80] | 96 | 64.29 [50.51-78.08] | |
| Mangodara | 69 | 63.07 [23.29-100] | 93 | 73.86 [48.83-98.89] | 102 | 91.31 [75.88-100] | |
| Nouna | 94 | 44.34 [32.87-55.80] | 87 | 78.68 [69.53-87.83] | 92 | 84.87 [76.83-92.92] | |
| Orodara | 97 | 31.95 [13.18-50.72] | 95 | 65.23 [60.22-70.25] | 89 | 85.21 [70.08-100] | |
| Seguenega | 93 | 24.88 [14.37-35.38] | 102 | 68.18 [57.44-78.92] | 95 | 83.04 [64.11-100] | |
| Solenzo | 103 | 14.64 [1.32-27.95] | 90 | 46.75 [12.98-80.53] | 97 | 59.39 [30.80-87.97] | |
| Soumousso | 109 | 16.52 [10.80-22.23] | 96 | 87.57 [82.76-92.37] | 93 | 92.30 [82.90-100] | |
| Diebougou | 104 | 29.45 [10.60-48.30] | 84 | 70.77 [47.97-93.57] | 111 | 92.44 [81.95-100] | |
| Karangasso-Vigué | 97 | 50.32 [9.61-91.03] | 100 | 72.82 [53.12-92.52] | 166 | 95.97 [90.74-100] | |
| Kaya | 93 | 11.82 [00.00-28.61] | 95 | 37.24 [16.88-57.60] | 116 | 45.56 [25.15-65.97] | |
| Boromo | 103 | 62.07 [38.19-85.94] | 88 | 84.69 [75.43-93.96] | 109 | 90.96 [86.38-95.54] | |
| Ouagadougou | 105 | 26.82 [12.40-41.23] | 121 | 73.25 [56.21-90.29] | 110 | 91.77 [79.27-100] | |

**Table 5. Percentage mortality of *An. gambiae* s.l. after exposure to permethrin at ×1, ×5, and ×10 the diagnostic concentration in WHO tube tests in 14 sites.**

| Sites | Permethrin diagnostic concentration | | | | | | Status |
|---|---|---|---|---|---|---|---|
| | 1x | | 5x | | 10x | | |
| | N | % mortality [CI] | N | % mortality [CI] | n | % mortality [CI] | |
| Kisumu strain | 100 | 100 | 100 | 100.0 | NA | NA | Susceptible colony |
| Gaoua | 93 | 13.57 [6.05-21.08] | 87 | 85.57 [77.64-93.50] | 87 | 94.97 [88.45-100] | High reistance intensity |
| Kampti | 109 | 23.38 [00.00-55.57] | 125 | 71.66 [58.10-85.22] | 103 | 100 | Moderate resistance intensity |
| Kongoussi | 96 | 10.10 [2.95-17.24] | 86 | 21.04 [7.11-34.96] | 90 | 95.60 [85.82-100] | High iresistance intensity |
| Mangodara | 93 | 49.12 [30.32-67.93] | 93 | 82.95 [66.36-99.55] | 104 | 98.20 [94.81-100] | Moderate intensity resistance |
| Seguenega | 92 | 8.92 [00.00-18.62] | 129 | 94.45 [91.33-97.57] | 105 | 95.30 [89.69-100] | High intensity resistance |
| Solenzo | 103 | 32.97 [20.92-45.02] | 108 | 92.87 [84.67-100] | 96 | 98 [93.40-100] | Moderate intensity resistance |
| Soumousso | 122 | 14.71 [8.31-21.11] | 108 | 80.10 [73.80-86.40] | 102 | 85.70 [69.55-100] | High intensity resistance |
| Kaya | 109 | 13.58 [00.00-27.22] | 109 | 89.86 [83.39-96.34] | 111 | 94.41 [86.83-100] | High intensity resistance |
| Boromo | 102 | 41.14 [15.40-66.88] | 116 | 72.45 [56.29-88.61] | 116 | 95.55 [89.90-100] | High intensity resistance |
| Diebougou | 109 | 53.55 [36.63-70.46] | 105 | 100 | NA | NA | Low intensity resistance |
| Nouna | 100 | 11.73 [6.66-16.81] | 120 | 78.58 [73.03-84.13] | 117 | 94.29 [87.79-100] | High intensity resistance |
| Orodara | 90 | 3.22 [00.00-9.16] | 99 | 57.47 [49.35-65.60] | 105 | 99.00 [96.22-100] | Moderate intensity resistance |
| Karangasso-Vigué | 114 | 43.57 [11.52-75.62] | 90 | 97.61 [93.11-100] | 96 | 100 | Moderate intensity resistance |
| Ouagadougou | 116 | 23.23 [5.40-41.05] | 112 | 84.69 [66.46-100] | 109 | 97.15 [91.31-100] | High intensity resistance |

**Table 6. Percentage mortality of *An. gambiae* s.l. after exposure to alpha-cypermethrin at ×1, ×5, and ×10 the diagnostic concentration in WHO tube tests in 14 sites.**

| Sites | Alphacypermethrin diagnostic concentration | | | | | | Status |
|---|---|---|---|---|---|---|---|
| | 1x N | % mortality [CI] | 5x N | % mortality [CI] | 10x n | % mortality [CI] | |
| Kisumu strain | 100 | 100 | 100 | 100 | NA | NA | Susceptible colony |
| Gaoua | 90 | 19.03 [16.75-21.32] | 97 | 65.18 [41.38-88.98] | 104 | 81.42 [73.84-89] | |
| Kampti | 106 | 65.99 [39.39-92.59] | 91 | 93.64 [82.21-100] | 83 | 96.29 [89.47-100] | |
| Kongoussi | 88 | 3.94 [00.00-16.51] | 103 | 37.30 [19.43-55.17] | 90 | 64.75 [56.26-73.25] | |
| Mangodara | 119 | 1.65 [00.00-4.77] | 111 | 43.25 [24.49-62.02] | 98 | 56.48 [34.44-78.51] | |
| Nouna | 79 | 11.80 [4.00-19.60] | 97 | 25.52 [13.96-37.08] | 95 | 45.83 [23.45-68.21] | |
| Orodara | 94 | 9.01 [00.00-19.00] | 89 | 16.67 [7.33-26] | 94 | 95.86 [86.85-100] | |
| Seguenega | 126 | 20.41 [13.26-27.55] | 119 | 41.82 [36.54-47.10] | 98 | 76.97 [67.60-86.33] | High resistance intensity |
| Solenzo | 90 | 15.82 [9.52-22.10] | 97 | 45.43 [27.83-63.02] | 93 | 67.18 [56.35-78.01] | |
| Soumousso | 112 | 41.53 [34.02-49.03] | 92 | 92.94 [86.85-99.02] | 99 | 95.99 [90.77-100] | |
| Diebougou | 108 | 12.20 [00.00-25.04] | 103 | 81.17 [64.00-98.34] | 105 | 93.34 [87.51-99.16] | |
| Kaya | 125 | 2.02 [00.00-8.47] | 108 | 29.31 [19.65-38.97] | 116 | 54.79 [49.02-60.56] | |
| Boromo | 107 | 40.44 [25.37-55.51] | 96 | 70.16 [47.28-93.04] | 111 | 71.42 [65.63-77.21] | |
| Karangasso-Vigué | 104 | 9.69 [1.60-17.79] | 163 | 69.54 [53.03-86.05] | 197 | 86.50 [76.15-96.84] | |
| Ouagadougou | 103 | 12.00 [00.00-30.21] | 141 | 66.48 [56.14-76.82] | 98 | 80.40 [64.11-96.68] | |

## 3.12 Distribution of Allele Frequencies of KDR (L1014F and L1014S) and *Ace-1R* Mutations

The allelic frequency of the West African kdr L1014F mutation showed high variation (Annex 9) in *An. gambiae* populations, but was particularly high in South Western sites (Gaoua, Kampti, Soumousso, Orodara and Mangodara) reaching frequencies between 72 percent and 98 percent. It was lower in some sites and was generally at moderate frequency in the Centre West and North with lowest frequency reported in Kaya with 30 percent. The allelic frequency of the West African kdr L1014F mutation was at relatively low frequencies in *An. coluzzii,* ranging from 0.313 in Kaya to 0.650 in Seguenega (for sites where a minimum of 10 *An. coluzzii* were tested). This mutation was also present within *An. arabiensis* populations at a frequency of 0.607 in Kampti up to 1.00 in Ouagadougou (for sites where a minimum of 10 *An. arabiensis* were tested

The kdr L1014S mutation was also found in *An. gambiae* populations, mostly in high frequencies in Gaoua, Solenzo, Kampti, and Nouna varying between 60 percent and 92 percent. The frequency was moderate in *An. coluzzii* populations at frequencies between 19 percent and 60 percent. This mutation was increasing in *An. arabiensis* particularly from the Southwest where it reached 60 percent in Gaoua. The two mutations occurred simultaneously within the same populations of *An. gambiae* s.l., indicating the implication of multiple resistance mechanisms (L1014F, L1014S and metabolic resistance). This possibly account for the moderate to high pyrethroid resistance intensity recorded at most sites tests and likelihood impact on efficacy of pyrethroid only LLINs.

The *ace-1*R G119S mutation was reported both in *An. gambiae* and *An. coluzzii* populations at increasing frequencies compared to 2018 in most sites tested, except in Kongoussi and Seguenega. Despite a general increase in *ace-1*R detection, the gene frequency was always less than 50 percent (Annex 10). The *Ace-1*R can cause resistance to organophosphates and carbamates; therefore it should be closely monitored in areas where IRS with pirimiphos-methyl (an organophosphate) is conducted. The only surprising finding is that the G119S mutation was recorded in *An. arabiensis* populations in Southwest and Centre West sites such as Kampti, Gaoua, Solenzo and Nouna. This mutation was first reported from *An. arabiensis* specimens collected in Bobo-Dioulasso (Dabire *et al*., 2014). ; Since then, no report of this mutation in *An. arabiensis* had been published. In the past two years, the frequency of the G119S mutation has increased across the country, particularly in *An. coluzzi* populations probably due to organophosphate use in semi-urban and urban agricultural activities. Additional molecular analysis (genes sequencing) could provide information on the spread of this mutation in Burkina Faso.

# 4. CONCLUSIONS

Cone bioassays with a susceptible insectary strain showed that Actellic CS, Fludora Fusion WP-SB and SumiShield WG lasted for at least seven months in all sites. The airborne fumigant effect was initially highly effective for all three formulations, but after three months, there was minimal fumigant effect.

The mean indoor and outdoor biting rates were consistently lower in Kongoussi (Actellic CS/SumiShield WG) compared to Seguenega (unsprayed); however, biting rates were far greater in Solenzo (SumiShield WG) than in Nouna (unsprayed). There was also no clear difference in biting rates for Kampti (Fludora Fusion WP-SB) and Gaoua (unsprayed). When broken down to sub-locations, there did appear to be a consistent impact of IRS in central sites compared to unsprayed areas, but not in rural sites where biting rates were higher.. The density of *An. gambiae* s.l. collected from PSC was generally lower in IRS sites compared to their paired unsprayed control sites, particularly in Kongoussi and Kampti.

Parity rates showed lower proportions of parous *An. gambiae* s.l. in Kongoussi sprayed with Actellic CS/SumiShield WG with 63 percent parous compared to 78 percent in Seguenega (unsprayed) and also lower in the sprayed site of Solenzo at 70 percent (SumiShield WG) compared to Nouna (unsprayed) at 78 percent. The mean sporozoite infection rates were high and did not differ between sprayed and unsprayed sites, ranging from 8.5 percent in Seguenega (unsprayed) to 14.1 percent in Solenzo (SumiShield WG). It would be useful to conduct a comparison of ELISA and PCR methods in 2020 to determine the most appropriate assay for sporozoite rates.

Overall, IRS appears to have an impact on the entomological inoculation rate in two out of the three sites, but malaria continues to be a considerable risk in all sites. IRS appears to be having some impact on malaria vector biting and resting proportions, particularly in central locations; however the impact in rural sites where vector densities are higher was less apparent. Overall, IRS appears to have an impact on EIR in two out of the three sites, but the malaria risk remains high in all the sites. In the two sites where PBO LLINs were distributed, the EIR was high, with 102 infectious bites per person in Karangasso-Vigué and 114 in Soumousso.

The insecticide susceptibility tests revealed that *An. gambiae* s.l. were resistant to all pyrethroids tested, but pre-exposure to PBO increased mosquito susceptibility to deltamethrin and permethrin in all sites tested, including Karangasso-Vigue and Soumouss where Permanet 3.0 nets were distributed in 2019. Pyrethroid resistance intensity was high in all sites for deltamethrin and alpha-cypermethrin and mostly moderate or high for permethrin. This high resistance intensity is probably affecting the performance of pyrethroid only LLINs Burkina Faso. Malaria vectors were susceptibility to chlorfenapyr at all sites. These results further support the decision for distribution of PBO and chlorfenapyr LLINs in Burkina Faso instead of pyrethroid only LLINs.

The susceptibility data showed that *An. gambiae* s.l. was fully susceptible to pirimiphos-methyl and clothianidin at all sites tested, including all three IRS locations. Therefore, these insecticide formulations (Actellic® 300CS, SumiShield 50WG and Fludora Fusion WP-SB) containing pirimiphos-methyl and clothianidin can continue to be used in Burkina Faso for IRS in rotations.

Overall, IRS appears to be having some impact on malaria vector biting and resting proportions particularly in central locations. However, there was little impact in rural sites where vector densities were higher.

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# 6. ANNEX

**Annex 1.Total number of *Culicidae* collected from June to December 2019 from indoor HLC in central and rural sites.**

| Study sites | *Culicidae* and sandflies caught by HLC (indoor & outdoor) collections | | | | | | | | | | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | *An. gambiae* s.l. | *An. nili* | *An. funestus* | *An. pharoensis* | *An. coustani* | Other *Anopheles* | *Culex* spp | *Aedes* spp | *Mansonia* | Phlebotomus spp | |
| Gaoua town (Unsprayed) | 1,863 | 686 | 19 | 77 | 8 | 2 | 777 | 151 | 22 | 2 | 3,607 |
| Gaoua rural (Unsprayed | 1,676 | 104 | 58 | 10 | 33 | 27 | 511 | 98 | 84 | 9 | 2,610 |
| Kampti town (Fludora® Fusion WP-SB) | 886 | 96 | 10 | 2 | 0 | 0 | 944 | 154 | 14 | 2 | 2,108 |
| Kampti rural (Fludora® Fusion WP-SB) | 2,471 | 111 | 60 | 1 | 5 | 1 | 40 | 26 | 32 | 1 | 2,748 |
| Nouna town (Unsprayed) | 467 | 1 | 0 | 2 | 0 | 1 | 2,295 | 55 | 4 | 14 | 2,839 |
| Nouna rural (Unsprayed) | 209 | 0 | 0 | 1 | 0 | 0 | 283 | 39 | 0 | 563 | 1,095 |
| Solenzo town (Shumishield WG) | 307 | 0 | 0 | 1 | 0 | 0 | 1,499 | 13 | 36 | 2 | 1,858 |
| Solenzo rural (Sumishield WG) | 2,482 | 1 | 22 | 4 | 0 | 1 | 320 | 1 | 5 | 2 | 2,838 |
| Seguenega town (Unsprayed) | 1,533 | 0 | 0 | 8 | 0 | 0 | 3,611 | 9 | 3 | 8 | **5,172** |
| Seguenega rural (Unsprayed) | 90 | 0 | 12 | 1 | 0 | 0 | 526 | 28 | 0 | 27 | 684 |
| Kongoussi town (Actellic CS) | 608 | 0 | 0 | 0 | 0 | 0 | 481 | 68 | 7 | 7 | 1,171 |
| Kongoussi rural (Sumishield WG) | 195 | 0 | 0 | 0 | 0 | 0 | 307 | 43 | 6 | 4 | 555 |
| Total number | **12,787** | 999 | 181 | 107 | 46 | 32 | 11,594 | 685 | 213 | 641 | **27,285** |

**Annex 2. Total number of *Culicidae* collected from June to December 2019 from PSC in central and rural sites.**

| Study sites | *Culicidae* and sandflies caught by PSC collection | | | | | | | | | | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | *An. gambiae* s.l. | *An. nili* | *An. pharoensis* | *An. funestus* | *An. coustani* | *Anophelesspp* | *Culex* spp | *Aedes* spp | *Mansonia spp* | *Phlebotomus* spp | |
| Gaoua town (Unsprayed) | 897 | 23 | 0 | 1 | 0 | 98 | 439 | 2 | 56 | 1 | 1,517 |
| Gaoua rural (Unsprayed | 580 | 1 | 0 | 40 | 0 | 0 | 200 | 1 | 25 | 35 | 882 |
| Kampti town Fludora® (Fusion WP-SB) | 85 | 0 | 0 | 0 | 0 | 5 | 217 | 33 | 2 | 17 | 359 |
| Kampti rural (Fludora® Fusion WP-SB) | 509 | 0 | 0 | 1 | 0 | 0 | 56 | 0 | 0 | 0 | 566 |
| Nouna town (Unsprayed) | 638 | 0 | 0 | 127 | 0 | 60 | 754 | 0 | 0 | 0 | 1,579 |
| Nouna rural (Unsprayed) | 841 | 0 | 0 | 27 | 0 | 0 | 331 | 1 | 0 | 68 | 1,268 |
| Solenzo town (Shumishield WG) | 312 | 0 | 0 | 0 | 0 | 1 | 938 | 0 | 0 | 2 | 1,253 |
| Solenzo rural (Sumishield WG) | 1,462 | 0 | 0 | 0 | 0 | 15 | 201 | 0 | 0 | 1 | 1,679 |
| Seguenega town (Unsprayed) | 1,148 | 0 | 0 | 1 | 0 | 16 | 950 | 3 | 6 | 3 | 2,127 |
| Seguenega rural (Unsprayed) | 278 | 0 | 0 | 4 | 0 | 0 | 351 | 0 | 0 | 5 | 638 |
| Kongoussi town (Sumishield WG) | 168 | 0 | 0 | 0 | 0 | 1 | 218 | 1 | 26 | 3 | 417 |
| Kongoussi rural (Sumishield WG) | 518 | 0 | 0 | 0 | 0 | 0 | 289 | 0 | 0 | 4 | 811 |
| Total | **7,436** | 24 | 0 | 201 | 0 | 196 | 4,944 | 41 | 115 | 139 | **13,096** |

**Annex 3. Nightly biting rate of *An. gambiae* s.l. collected by HLC (indoor + outdoor) collection from June to December 2019 in the IRS and control urban and rural sites**

| **Overall biting rate per night of *An. gambiae* s.l.from central sites** | | | | | | |
|---|---|---|---|---|---|---|
| **Months** | **Gaoua central (Unsprayed)** (N=1863) | **Kampti central Fludora® Fusion WP-SB** (N=886) | **Nouna central (Unsprayed)** (N=467) | **Solenzo central Shumishield WG** (N=307) | **Seguenega central (Unsprayed)** (N=1533) | **Kongoussi central Actellic CS** (N=608) |
| June | 6.88 | 1.88 | 0.56 | 0.03 | 0.81 | 0.03 |
| July | 9.22 | 3.41 | 1.91 | 0.53 | 8.97 | 1.84 |
| August | 4.41 | 5.59 | 4.44 | 2.47 | 22.69 | 14.00 |
| September | 24.38 | 9.03 | 4.91 | 6.09 | 7.63 | 1.69 |
| October | 12.41 | 7.34 | 2.34 | 0.44 | 7.31 | 1.44 |
| November | 0.94 | 0.44 | 0.44 | 0.03 | 0.47 | 0 |
| December | 0 | 0 | 0 | 0 | 0.03 | 0 |
| **Mean biting rate per night** | **8.32** | **4.00** | **2.08** | **1.37** | **6.84** | **2.71** |

| **Overall biting rate per night of *An. gambiae* s.l. from rural sites** | | | | | | |
|---|---|---|---|---|---|---|
| **Months** | **Gaoua rural (Unsprayed)** (N=1676) | **Kampti rural Fludora® Fusion WP-SB** (N=2471) | **Nouna rural (Unsprayed)** (N=209) | **Solenzo rural Sumishield WG** (N=2482) | **Seguenega rural (Unsprayed) (N=90)** | **Kongoussi rural Sumishield WG** (N=195) |
| June | 4.22 | 11.16 | 0.16 | 0 | 0.03 | 0 |
| July | 6.81 | 2.09 | 0.94 | 3.63 | 0.59 | 2.69 |
| August | 6.03 | 11.84 | 0.66 | 12.47 | 1.41 | 2.47 |
| September | 28.09 | 38.78 | 4.53 | 53.53 | 0.34 | 0.69 |
| October | 6.84 | 10.09 | 0.22 | 5.94 | 0.44 | 0.06 |
| November | 0.38 | 3.25 | 0.03 | 1.91 | 0 | 0.19 |
| December | 0 | 0 | 0 | 0.09 | 0 | 0 |
| **Mean biting rate per night** | **7.48** | **11.03** | **0.93** | **11.08** | **0.40** | **0.87** |

**Annex 4. Mean infection rate of *An. gambiae* s.l. collected by HLC (indoor + outdoor) collection from June to December 2019 in the IRS and control urban and rural sites.**

| Infection rate of *An. gambiae* s.l. from central sites | | | | | | |
|---|---|---|---|---|---|---|
| **Months** | **Gaoua central (Unsprayed)** (N=300) | **Kampti central Fludora® Fusion WP-SB** (N=228) | **Nouna central (Unsprayed)** (N=175) | **Solenzo central Shumishield WG** (N=185) | **Seguenega central (Unsprayed)** (N=264) | **Kongoussi central Actellic CS** (N=185) |
| June | 6 | 6 | 10 | 0 | 11.42 | 0 |
| July | 4 | 2.27 | 8 | 5.12 | 10 | 6 |
| August | 16 | 30 | 4 | 20 | 6 | 0 |
| September | 14 | 18 | 24 | 8 | 12 | 28 |
| October | 2 | 8 | 6 | 7.5 | 8 | 14.28 |
| November | 12 | 14.29 | 2.86 | 25 | 0 | 0 |
| December | 0 | 0 | 0 | 0 | 12.5 | 0 |
| **Mean infection rate** | **7.71** | **11.22** | **7.84** | **9.37** | **8.56** | **6.90** |

| Infection rate of *An. gambiae* s.l. from rural sites | | | | | | |
|---|---|---|---|---|---|---|
| **Months** | **Gaoua rural (Unsprayed)** (N=265) | **Kampti rural Fludora® Fusion WP-SB** (N=298) | **Nouna rural (Unsprayed)** (N=232) | **Solenzo rural Sumishield WG** (N=254) | **Seguenega rural (Unsprayed)** (N=139) | **Kongoussi rural Sumishield WG** (N=214) |
| June | 6 | 12 | 2.7 | 33.33 | 0 | 18.42 |
| July | 12 | 36 | 16 | 10 | 12.5 | 2 |
| August | 36 | 10 | 27.77 | 10 | 10 | 14 |
| September | 24 | 18 | 18 | 2 | 16.67 | 34 |
| October | 18 | 2 | 18 | 14 | 18 | 15.38 |
| November | 20 | 33.33 | 18.51 | 0 | 0 | 15.38 |
| December | 0 | 0 | 0 | 33.33 | 0 | 0 |
| **Mean infection rate** | **16.57** | **15.90** | **14.43** | **14.67** | **8.17** | **14.17** |

**Annex 5. Infection rates of *An. gambiae* s.l. females to *P. falciparum* from indoors and outdoors collections in PBO-LLINs sites.**

| Months | Karangasso-Vigué | Soumousso |
|---|---|---|
| **Sporozoite rate of *An. gambiae* s.l. from HLC indoor collections** | | |
| June | 23.53 (4/17) | 15.79 (6/38) |
| July | 0 (0/14) | 8.33 (3/36) |
| August | 6.45 (2/31) | 10.71 (3/28) |
| September | 8.82 (3/34) | 36.84 (7/19) |
| October | 13.04 (3/23) | 22.22 (6/27) |
| November | 29.63 (8/27) | 16.67 (6/36) |
| December | 0 (0/0) | 0 (0/0) |
| Total | **13.69% (20/146)** | **16.85% (31/184)** |
| **Sporozoite rate of *An. gambiae* s.l. from HLC outdoor collections** | | |
| June | 0 (0/6) | 8.33 (1/12) |
| July | 0 (0/23) | 7.14 (1/14) |
| August | 5.26 (1/19) | 0 (0/22) |
| September | 6.25 (1/16) | 9.68 (3/31) |
| October | 7.41 (2/27) | 13.04 (3/23) |
| November | 28.57 (4/14) | 27.27 (3/11) |
| December | 0 (0/0) | 0 (0/0) |
| Total | **7.62% (8/105)** | **11.50% (13/113)** |
| Overall indoor + outdoor | **10.94% (28/256)** | **14.81% (44/297)** |

**Annex 6. Nightly entomological inoculation rate of *An. gambiae s.l.* collected by HLC (indoor + outdoor) collection from June to December 2019 in the IRS and control urban and rural sites.**

| Overall EIR per night of *An. gambiae* s.l. from central sites | | | | | | |
|---|---|---|---|---|---|---|
| **Months** | **Gaoua central (Unsprayed)** | **Kampti central Fludora® Fusion WP-SB** | **Nouna central (Unsprayed)** | **Solenzo central Shumishield WG** | **Seguenega central (Unsprayed)** | **Kongoussi central Actellic CS** |
| June | 0.41 | 0.11 | 0.06 | 0 | 0.09 | 0 |
| July | 0.37 | 0.08 | 0.15 | 0.03 | 0.90 | 0.11 |
| August | 0.71 | 1.68 | 0.18 | 0.49 | 1.36 | 0 |
| September | 3.41 | 1.63 | 1.18 | 0.49 | 0.92 | 0.47 |
| October | 0.25 | 0.59 | 0.14 | 0.03 | 0.59 | 0.21 |
| November | 0.11 | 0.06 | 0.01 | 0.01 | 0 | 0 |
| December | 0 | 0 | 0 | 0 | 0.01 | 0 |
| **Mean EIR per night** | **0.75** | **0.59** | **0.25** | **0.15** | **0.55** | **0.11** |

| Overall EIR per night of *An. gambiae* s.l. from rural sites | | | | | | |
|---|---|---|---|---|---|---|
| **Months** | **Gaoua rural (Unsprayed )** | **Kampti rural Fludora® Fusion WP-SB** | **Nouna rural (Unsprayed)** | **Solenzo rural Sumishield WG** | **Seguenega rural (Unsprayed)** | **Kongoussi rural Sumishield WG** |
| June | 0.25 | 1.34 | 0.01 | 0.00 | 0.00 | 0.00 |
| July | 0.82 | 0.75 | 0.15 | 0.36 | 0.07 | 0.05 |
| August | 2.17 | 1.18 | 0.18 | 1.25 | 0.14 | 0.35 |
| September | 6.74 | 6.98 | 0.82 | 1.07 | 0.06 | 0.23 |
| October | 1.23 | 0.20 | 0.04 | 0.83 | 0.08 | 0.01 |
| November | 0.08 | 1.08 | 0.01 | 0.00 | 0.00 | 0.03 |
| December | 0 | 0 | 0 | 0.03 | 0.00 | 0.00 |
| **Mean EIR per night** | **1.61** | **1.65** | **0.17** | **0.51** | **0.05** | **0.10** |

**Annex 7. Monthly Entomological inoculation rate of *An. gambiae* s.l. collected by HLC (indoor + outdoor) collection from June to December 2019 in the IRS and control town and rural sites.**

| Overall EIR per month of *An. gambiae* s.l. from central sites | | | | | | |
|---|---|---|---|---|---|---|
| **Months** | **Gaoua central (Unsprayed)** | **Kampti central Fludora® Fusion WP-SB** | **Nouna central (Unsprayed)** | **Solenzo central Shumishield WG** | **Seguenega central (Unsprayed)** | **Kongoussi central Actellic CS** |
| June | 12.38 | 3.38 | 1.69 | 0.00 | 2.78 | 0.00 |
| July | 11.06 | 2.32 | 4.58 | 0.82 | 26.91 | 3.32 |
| August | 21.15 | 50.34 | 5.33 | 14.81 | 40.84 | 0.00 |
| September | 102.38 | 48.77 | 35.33 | 14.63 | 27.45 | 14.18 |
| October | 7.44 | 17.63 | 4.22 | 0.98 | 17.55 | 6.16 |
| November | 3.38 | 1.88 | 0.38 | 0.23 | 0.00 | 0.00 |
| December | 0 | 0.00 | 0.00 | 0 | 0.12 | 0.00 |
| **Mean EIR per month** | **22.54** | **17.76** | **7.36** | **4.50** | **16.52** | **3.38** |

| Overall EIR per month of *An. gambiae* s.l. from rural sites | | | | | | |
|---|---|---|---|---|---|---|
| **Months** | **Gaoua rural (Unsprayed)** | **Kampti rural Fludora® Fusion WP-SB** | **Nouna rural (Unsprayed)** | **Solenzo rural Sumishield WG** | **Seguenega rural (Unsprayed)** | **Kongoussi rural Sumishield WG** |
| June | 7.59 | 40.16 | 0.13 | 0.00 | 0.00 | 0.00 |
| July | 24.53 | 22.61 | 4.50 | 10.88 | 2.23 | 1.61 |
| August | 65.14 | 35.53 | 5.47 | 37.41 | 4.22 | 10.37 |
| September | 202.28 | 209.42 | 24.47 | 32.12 | 1.72 | 7.01 |
| October | 36.96 | 6.06 | 1.18 | 24.94 | 2.36 | 0.29 |
| November | 2.25 | 32.50 | 0.17 | 0.00 | 0.00 | 0.87 |
| December | 0.00 | 0.00 | 0.00 | 0.94 | 0.00 | 0.00 |
| **Mean EIR per month** | **48.39** | **49.47** | **5.13** | **15.18** | **1.50** | **2.88** |

**Annex 8. Monthly entomological inoculation rate of *An. gambiae* s.l. collected by HLC indoor and outdoor collection from June to December 2019 in PBO-LLINs sites.**

| Months | Karangasso-Vigué | Soumousso |
|---|---|---|
| **EIR per month of *An. gambiae* s.l. indoor** | | |
| June | 0.31 | 8.17 |
| July | 0.00 | 15.26 |
| August | 8.82 | 19.88 |
| September | 16.89 | 133.45 |
| October | 40.49 | 26.50 |
| November | 5.49 | 3.11 |
| December | 0.00 | 0.00 |
| **EIR indoors for 7 months** | **72.00** | **206.37** |
| **EIR per month of *An. gambiae* s.l. outdoor** | | |
| June | 0.00 | 1.70 |
| July | 0.00 | 7.55 |
| August | 7.62 | 4.40 |
| September | 14.53 | 32.23 |
| October | 39.18 | 13.94 |
| November | 8.04 | 5.58 |
| December | 0.00 | 0.00 |
| **EIR outdoors for 7 months** | **69.36** | **65.41** |
| **OVERALL EIR (indoor + outdoor)** | | |
| **Overall EIR (indoor + outdoor) for 7 months** | **141.36** | **271.78** |

**Annex 9. Distribution and frequency of the L1014F and L1014S knockdown resistance (kdr) alleles of *An. gambiae* s.l. from PMI and NMCP sites in Burkina Faso.**

| Species | Sites | N | Genotypes (kdr west) 1014L/1014L | 1014L/1014F | 1014F/1014F | f(L1014F) | p(HW) | N | Genotypes (kdr east) 1014L/1014L | 1014L/1014S | 1014S/1014S | f(1014S) | p(HW) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *An. arabiensis* | **Kampti** | 28 | 5 | 12 | 11 | **0.607** | 0.6971 | 26 | 5 | 19 | 2 | **0.411** | 0.0570 |
| | **Gaoua** | 19 | 4 | 6 | 9 | **0.632** | 0.1688 | 20 | 2 | 12 | 6 | **0.60** | 0.3830 |
| | **Solenzo** | 9 | 6 | 1 | 2 | **0.278** | 0.0585 | 9 | 7 | 1 | 1 | **0.167** | 0.1773 |
| | **Nouna** | 5 | 3 | 2 | 0 | **0.200** | 1.0000 | 5 | 4 | 1 | 0 | **0.100** | - |
| | **Kongoussi** | - | - | - | - | **-** | - | - | - | - | - | **-** | - |
| | **Seguenega** | - | - | - | - | **-** | - | - | - | - | - | **-** | - |
| | **Orodara** | 4 | 0 | 0 | 4 | **1.000** | - | 4 | 4 | 0 | 0 | **0.000** | - |
| | **Soumousso** | 6 | 6 | 0 | 0 | **0.000** | - | 6 | 4 | 0 | 2 | **0.333** | 0.0304 |
| | **Ouagadougou** | 10 | 0 | 0 | 10 | **1.000** | - | 10 | 9 | 1 | 0 | **0.050** | - |
| | **Boromo** | 3 | 0 | 0 | 3 | **1.000** | - | 3 | 3 | 0 | 0 | **0.000** | - |
| | **Mangodara** | - | - | - | - | **-** | - | - | - | - | - | **-** | - |
| | **Kaya** | - | - | - | - | **-** | - | - | - | - | - | **-** | - |
| *An. coluzzii* | **Kampti** | 9 | 2 | 5 | 2 | **0.500** | 1.0000 | 9 | 5 | 2 | 2 | **0.333** | 0.1705 |
| | **Gaoua** | 3 | 1 | 0 | 2 | **0.667** | 0.2008 | 3 | 0 | 2 | 1 | **0.667** | 1.0000 |
| | **Solenzo** | 26 | 5 | 11 | 10 | **0.596** | 0.6838 | 27 | 11 | 10 | 6 | **0.407** | 0.2439 |
| | **Nouna** | 43 | 15 | 12 | 16 | **0.512** | 0.0055 | 43 | 27 | 7 | 9 | **0.291** | 0.0001 |
| | **Kongoussi** | 47 | 2 | 29 | 16 | **0.649** | 0.0251 | 47 | 22 | 10 | 15 | **0.426** | 0.0002 |
| | **Seguenega** | 10 | 1 | 5 | 4 | **0.650** | 1.0000 | 10 | 3 | 3 | 4 | **0.550** | 0.2450 |
| | **Orodara** | 2 | 1 | 1 | 0 | **0.250** | - | 2 | 0 | 0 | 2 | **1.000** | - |
| | **Soumousso** | 1 | 0 | 0 | 1 | **1.000** | - | 1 | 1 | 0 | 0 | **0.000** | - |
| | **Ouagadougou** | - | - | - | - | **-** | - | - | - | - | - | **-** | - |
| | **Boromo** | 10 | 4 | 3 | 3 | **0.450** | 0.2448 | 10 | 7 | 3 | 0 | **0.150** | 1.0000 |
| | **Mangodara** | 7 | 1 | 4 | 2 | **0.571** | 1.0000 | 7 | 0 | 0 | 7 | **1.000** | - |
| | **Kaya** | 42 | 17 | 17 | 8 | **0.393** | 0.3381 | 50 | 38 | 8 | 4 | **0.190** | 0.0238 |
| *An. gambiae* | **Kampti** | 13 | 0 | 2 | 11 | **0.923** | 1.0000 | 13 | 1 | 0 | 12 | **0.923** | 0.0411 |
| | **Gaoua** | 27 | 2 | 4 | 21 | **0.852** | 0.0712 | 27 | 2 | 3 | 22 | **0.870** | 0.0362 |
| | **Solenzo** | 11 | 4 | 1 | 6 | **0.591** | 0.0097 | 11 | 4 | 0 | 7 | **0.636** | 0.0012 |
| | **Nouna** | 2 | 1 | 0 | 1 | **0.500** | 0.3303 | 2 | 0 | 1 | 1 | **0.750** | - |
| | **Kongoussi** | 2 | 1 | 1 | 0 | **0.250** | - | 3 | 1 | 2 | 0 | **0.333** | 1.0000 |
| | **Seguenega** | 36 | 8 | 23 | 5 | **0.458** | 0.1784 | 38 | 14 | 18 | 6 | **0.395** | 1.0000 |
| | **Orodara** | 44 | 2 | 4 | 38 | **0.909** | 0.0271 | 44 | 43 | 0 | 1 | **0.023** | 0.0115 |
| | **Soumousso** | 43 | 1 | 0 | 42 | **0.977** | 0.0110 | 42 | 41 | 0 | 1 | **0.024** | 0.0108 |
| | **Ouagadougou** | 40 | 3 | 0 | 37 | **0.925** | 0.0000 | 40 | 23 | 14 | 3 | **0.250** | 0.6793 |
| | **Boromo** | 37 | 13 | 10 | 14 | **0.514** | 0.0066 | 34 | 23 | 10 | 1 | **0.176** | 1.0000 |
| | **Mangodara** | 43 | 8 | 8 | 27 | **0.721** | 0.0006 | 43 | 39 | 0 | 4 | **0.093** | 0.0000 |
| | **Kaya** | 8 | 3 | 5 | 0 | **0.312** | 0.4859 | 8 | 6 | 2 | 0 | **0.125** | 1.0000 |

N: number of mosquitoes; f(1014F) : frequency of the 1014F resistant *kdr* allele; f(1014S) : frequency of the 1014S resistant *kdr* allele; p(HW): probability of the exact test for goodness of fit to Hardy-Weinberg equilibrium; '-': not determine

**Annex 10. Allelic and genotypic frequencies at the ace-1 locus in *An. gambiae* s.l. populations from PMI sites in Burkina Faso.**

| | | | Genotypes | | | | |
|---|---|---|---|---|---|---|---|
| Species | Sites | N | 119G 119G | 119G 119S | 119S 119S | f(119S) | p(HW) |
| *An. arabiensis* | **Kampti** | 17 | 11 | 5 | 1 | 0.206 | 0.5364 |
| | **Gaoua** | 12 | 8 | 4 | 0 | 0.167 | 1.0000 |
| | **Solenzo** | 5 | 2 | 3 | 0 | 0.300 | 1.0000 |
| | **Nouna** | 3 | 2 | 1 | 0 | 0.167 | - |
| | **Kongoussi** | - | - | - | - | - | - |
| | **Seguenega** | - | - | - | - | - | - |
| | **Orodara** | - | - | - | - | - | - |
| | **Soumousso** | 4 | 4 | 0 | 0 | 0.000 | - |
| | **Ouagadougou** | 14 | 14 | 0 | 0 | 0.000 | - |
| | **Boromo** | 2 | 2 | 0 | 0 | 0.000 | - |
| | **Mangodara** | - | - | - | - | - | - |
| | **Kaya** | - | - | - | - | - | - |
| *An. coluzzii* | **Kampti** | 3 | 1 | 2 | 0 | 0.333 | 1.0000 |
| | **Gaoua** | 2 | 2 | 0 | 0 | 0.000 | - |
| | **Solenzo** | 31 | 14 | 17 | 0 | 0.274 | 0.0674 |
| | **Nouna** | 42 | 32 | 9 | 1 | 0.131 | 0.5285 |
| | **Kongoussi** | 35 | 35 | 0 | 0 | 0.000 | - |
| | **Seguenega** | 31 | 31 | 0 | 0 | 0.000 | - |
| | **Orodara** | - | - | - | - | - | - |
| | **Soumousso** | 1 | 0 | 1 | 0 | 0.500 | - |
| | **Ouagadougou** | 2 | 2 | 0 | 0 | 0.000 | - |
| | **Boromo** | 12 | 9 | 3 | 0 | 0.125 | 1.0000 |
| | **Mangodara** | 5 | 1 | 4 | 0 | 0.400 | 0.4301 |
| | **Kaya** | 40 | 30 | 9 | 1 | 0.138 | 0.5530 |
| *An. gambiae* | **Kampti** | 30 | 12 | 18 | 0 | 0.300 | 0.0301 |
| | **Gaoua** | 34 | 16 | 17 | 1 | 0.279 | 0.3874 |
| | **Solenzo** | 10 | 4 | 6 | 0 | 0.300 | 0.4788 |
| | **Nouna** | 4 | 2 | 2 | 0 | 0.250 | 1.0000 |
| | **Kongoussi** | 15 | 15 | 0 | 0 | 0.000 | - |
| | **Seguenega** | 17 | 17 | 0 | 0 | 0.000 | - |
| | **Orodara** | 48 | 17 | 28 | 3 | 0.354 | 0.1085 |
| | **Soumousso** | 44 | 34 | 9 | 1 | 0.125 | 0.5163 |
| | **Ouagadougou** | 29 | 24 | 5 | 0 | 0.086 | 1.0000 |
| | **Boromo** | 33 | 26 | 5 | 2 | 0.136 | 0.0829 |
| | **Mangodara** | 45 | 12 | 31 | 2 | 0.389 | 0.0041 |
| | **Kaya** | 10 | 8 | 1 | 1 | 0.150 | 0.1593 |

N: number of mosquitoes; f (119S): frequency of the 119S resistant *ace1* allele; p(HW): probability of the exact test for goodness of fit to Hardy-Weinberg equilibrium; '-': not determine