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PMI VECTORLINK BURKINA FASO ENTOMOLOGY FINAL REPORT

JANUARY – DECEMBER 2018

SUBMITTED APRIL 01, 2019

Recommended Citation: The PMI VectorLink Project. Burkina Faso Entomology Final Report: January – December 2018. Rockville, MD. The PMI VectorLink Project, Abt Associates Inc/ Institut de Recherche en Sciences de la Santé / Health Sciences Research Institute (IRSS).

Contract: AID-OAA-I-17-00008

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

Submitted to: United States Agency for International Development/PMI

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ACRONYMS

ACE-1	Insensitive Acetylcholinesterase Gene
ACT	Artemisinin-based Combination Therapy
ANOVA	Analysis of Variance
CDC	Centers for Disease Control and Prevention
CTAB	Cetyl Trimethylammonium Bromide
DNA	Deoxyribonucleic Acid
EC	Emulsifiable Concentrate
EIR	Entomological Inoculation Rate
ELISA	Enzyme-Linked Immunosorbent Assay
GPIRM	Global Plan for Insecticide Resistance Management
HLC	Human Landing Catch
HBR	Human Biting Rate
IgG	Immunoglobulin G
IR	Infection Rate
IRS	Indoor Residual Spraying
IRSS	Institut de Recherche en Sciences de la Santé / Health Sciences Research Institute
KDR	Knock Down Resistance
LLIN	Long Lasting Insecticide Treated Bednet
NMCP	National Malaria Control Program
PBO	Piperonyl Butoxide
PCR	Polymerase Chain Reaction
PMI	United States President's Malaria Initiative
PSC	Pyrethrum Spray Catch
RDT	Rapid Diagnostic Test
SOP	Standard Operating Procedure
WHO	World Health Organization

EXECUTIVE SUMMARY

This report presents the field evaluations that the United States President's Malaria Initiative (PMI) VectorLink Burkina Faso program conducted to monitor malaria vectors and the efficacy of indoor residual spraying (IRS) application with pirimiphos methyl (Actellic 300CS) and clothianidin (SumiShield 50 WG) insecticides in selected geographical areas in Burkina Faso. Additionally, the data of monitoring and susceptibility testing conducted in National Malaria Control Program (NMCP) sentinel sites were also reported. This report summarizes entomological data collected from June to December 2018 and details the main entomological parameters evaluated.

IRS took place in June 2018, where the VectorLink Project sprayed Actellic 300CS insecticide in Kongoussi and Solenzo, and SumiShield 50 WG in Kampti and Solenzo. The paired control sites were Seguenega for Kongoussi, Gaoua for Kampti and Nouna for Solenzo.

Results from World Health Organization (WHO) cone bioassays performed in June (T0) illustrate that the spray teams applied IRS effectively, with 100 percent mosquito mortality recorded within one week of spraying. Cone bioassays with the *An. gambiae* Kisumu susceptible insectary strain showed that both Actellic CS and SumiShield 50 WG lasted for at least six months (T0-T6) in all sites and on all surfaces. The monthly evaluation did not indicate reduction in efficacy except in Kongoussi (Actellic) in the latest months where reduced mortalities were observed with wild strains of *An. gambiae* s.l. originating from the same locality both on mud and concrete walls. However, tests with wild *An. gambiae* s.l. in Kongoussi produced lower mortality rates (50-56% particularly on concrete walls five and six months after spraying), which is an indication of potential pirimiphos-methyl resistance in this site.

The main entomological parameters of transmission were significantly lower in sprayed site of Kampti compared to the control site in Gaoua. From June to December 2018, *An. gambiae* s.l. biting rates were three times lower in Kampti than Gaoua and the overall sporozoite rate was 6.95% in the IRS site compared to 13.02% in unsprayed Gaoua. However, despite good efficacy in cone bioassays, biting densities of *An. gambiae* s.l. were similar in Solenzo and Kongoussi compared to their respective control sites. Sporozoite rates were lower in Kampti, Solenzo, and Kongoussi towns, compared to their paired controls; they appear to indicate that IRS with SumiShield WG and Actellic CS is having a positive impact on infection rates (assuming that paired control areas were comparable).

The entomological inoculation rate (EIR) from June to December was significantly lower in Kampti compared at 19 infectious bites per person per night indoors, compared to 142 in Gaoua. The EIR was also lower in Kongoussi than unsprayed Seguenega, except in September when there was a large peak in Kongoussi. This situation was also observed in 2017 in the same site with a different insecticide. While IRS appears to be having some impact on malaria transmission, particularly by reducing sporozoite rates, the high biting rates and EIR in some IRS sites are concerning.

The WHO tube tests revealed resistance in *An. gambiae* s.l. for all pyrethroids tested and moderate resistance to bendiocarb in all sites tested. Pre-exposure to the synergist piperonyl butoxide (PBO) increased mosquito susceptibility to deltamethrin, suggesting that PBO long-lasting insecticidal nets (LLINs) may provide greater control of malaria vectors in Burkina Faso than conventional pyrethroid LLINs. Susceptibility to chlorfenapyr and clothianidin, new insecticides for vector control, was recorded

at all sites. This suggests that Interceptor G2 LLINs (containing chlorfenapyr + alphacypermethrin) are also likely to provide greater control than conventional pyrethroid LLINs in Burkina Faso.

Full susceptibility of *An. gambiae* s.l. to pirimiphos-methyl and clothianidin was observed at all sites in 2018. This is in contrast to the 2017 results, which showed pirimiphos-methyl resistance in Gaoua, Kampti, Mangodara, and Solenzo. WHO papers from 2017 and 2018 have been sent to the WHO collaborating center in Belgium for HPLC analysis to determine whether those filter papers were under-dosed. However, cone bioassays with wild *An. gambiae* s.l. indicated possible pirimiphos-methyl resistance in Kongoussi (lower mortality than the susceptible insectary strain).

While both SumiShield WG and Actellic CS produced good cone bioassay results, the impact of both insecticides on entomological indices was limited and there was no clear difference between the two formulations. Due to the apparent presence of pirimiphos-methyl resistance in Kongoussi and potential resistance in other locations, it would be prudent to conduct IRS with clothianidin-based insecticides and/or deploy next generation nets such as PBO or G2 LLINs in 2019. While IRS appears to be having some impact on malaria transmission, particularly by reducing sporozoite rates, the high biting rates and EIRs in some IRS sites are concerning. Susceptibility testing indicates that the NMCP has a series of options for LLIN distribution to combat pyrethroid resistance.

INTRODUCTION

The World Health Organization (WHO) has reported 219 million malaria cases and 435,000 deaths worldwide in 2017 (WHO, 2018). Malaria is endemic in Burkina Faso, and the recent malaria 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 is jeopardising the effectiveness of this strategy (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 (Nguessan et al., 2007; Toé et al., 2015).

In this context, indoor residual spraying (IRS) with non-pyrethroid insecticides could be an important strategy to effectively control pyrethroid-resistant vectors and to break down malaria transmission sustained by resistant *An. gambiae* s.l. A study conducted in 2013 in Diebougou, an endemic malaria transmission area located in Bougouriba Province (southwestern Burkina Faso), showed that IRS using bendiocarb, an insecticide of the carbamate class, resulted in a substantial decrease in malaria transmission. However, the residual efficacy did not reach three months (Dabiré et al*.,* report of field activities). Due to the short longevity and lack of sustained funding, bendiocarb IRS was not extended to other regions and was also discontinued in Bougouriba after the pilot of 2011-12.

The NMCP recently updated its national malaria strategic plan covering the period of 2016-2020 and recommended that non-pyrethroid 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, IRS was included as a priority vector control strategy to be implemented in 2018. Before the implementation of IRS in Burkina Faso, to achieve the objective of the Global Plan for Insecticide Resistance Management (GPIRM), it was crucial to collect baseline entomological data in 2017, prior to IRS in 2018, to accurately determine the impact of this intervention. The VectorLink Burkina Faso team conducted surveys to monitor vector bionomics and insecticide susceptibility during the period of high malaria transmission (June to December, 2017) in three eco-climatic zones (sudanian, sudano-sahelian and sahelian) to monitor prospective IRS sites and their respective neighboring control sites.. After conducting IRS in June 2018 in Kampti, Solenzo and Kongoussi districts, the team carried out monthly entomolgogical monitoring to gather key information to evaluate the impact of IRS in malaria transmission. Nationwide resistance data was also collected and shared with the NMCP to assist with vector control planning. This report provides the key results of six months' data collection from June to December 2018 and compares to the data gathered the previous year in 2017 to measure the impact of the first application of IRS in a large scale in 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 or Sumishield 50 WG and to determine the impact of IRS in 2018.

The specific objectives of the program were to:

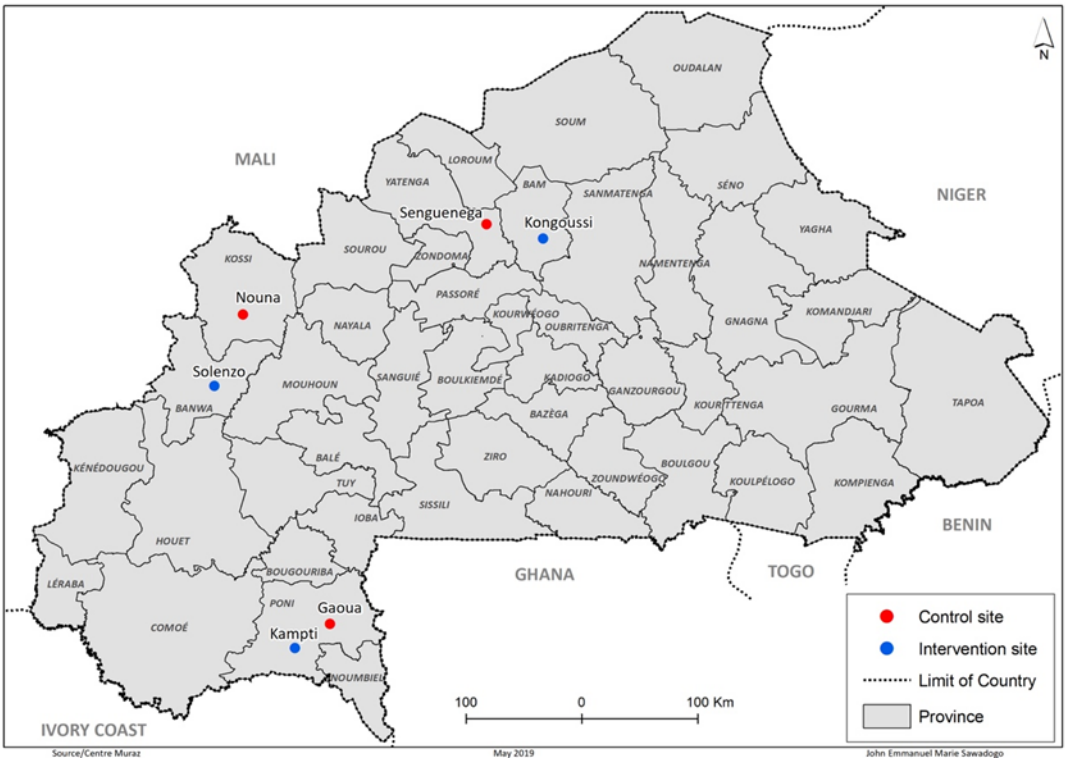
- Collect detailed information on mosquito biting rates, biting times, indoor resting densities, seasonality, and parity rates of malaria vectors in both IRS sites and their adjacent control unsprayed sites.
- Determine the impact of IRS on entomological outcomes by comparing the 2017 data (when all sites were unsprayed) with 2018 results.
- 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 (with and without pre-exposure to the synergist piperonyl-butoxide (PBO)), bendiocarb 0.1 percent, and pirimiphos-methyl 0.25 percent in 21 sentinel sites selected by NMCP.
- Determine the intensity of insecticide resistance to deltamethrin using te Centers for Disease Control and Prevention (CDC) bottle assays nationwide (16 sentinel sites among those adopted by the NMCP).
- Provide technical assistance to the NMCP in the development of its national resistance monitoring plan.

I. METHODOLOGY

1.1 STUDY AREA

The VectorLink Burkina Faso team carried out entomological monitoring during the high transmission season from June to December 2018, right after IRS implementation. The team conducted monthly mosquito collections in three sprayed sites and three unsprayed sites to measure entomological parameters of malaria transmission. Cone bioassays were conducted on sprayed walls in the three IRS districts to measure the quality of IRS and residual efficacy. Figure 1 shows the location of the three sprayed and three unsprayed sites used for monthly entomological monitoring. The six sites are located across the three ecological zones of Burkina Faso: Sudanese (West), Sudan-Sahelian (Center-West) and Sahelian (North) zones. The three intervention sites included Kampti (10°7′60″N, 3°27'0"W, province of Poni, South-West Region), Solenzo (12°11'N, 4°05'W, province of Banwa, Boucle du Mouhoun Region) and Kongoussi (13°19′33″N, 1°32′5″E, province of Bam, Central North Region). The unsprayed control sites were Gaoua (10°60'N; 4°70'W, province of Poni, south-west Region), Nouna (12°37'N, 3°55°W, province of Kossi, Boucle du Mouhoun centre West Region) and Seguenega (13°15′30″N, 1°58′1″E, province of Yatenga, North Region). The sprayed sites were paired with neighboring unsprayed sites (approx 50 km away) that had similar characteristics.

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



1.2 MOSQUITO SAMPLING

Anopheles mosquitoes were sampled monthly from June to December during the rainy season using human landing catches (HLC) and pyrethrum spray catches (PSC). Larval collections of *An. gambiae* s.l. were conducted during the rainy season and reared to adults for insecticide resistance monitoring.

1.2.1 HUMAN LANDING CATCH (HLC)

Human landing catches were conducted monthly from June to December 2018. The same four houses were used for HLC sampling in each sprayed and control site for two consecutive nights per month. The team deployed four collectors (in two shifts) per house per night so that at all times there was one collector indoors and one outdoors. Indoor HLC was conducted in the living room of the house while outdoor HLC was within five meters of the front door. The collection period lasted from 8 p.m. to 9 a.m. and was divided into two shifts, so that one person managed each station for half the collection period before being replaced by another person. Each collector sat on a stool and exposed his lower legs for mosquitoes to land on. The collector monitored mosquitoes as they landed and captured them with mouth aspirators. Collectors placed mosquitoes in a paper cup and provided them with sugar solution. The collector used a new cup each hour to record the time of biting.

The mosquito collectors conducting the HLC were volunteers recruited from the community, who had consented and were provided with requisite training. Collectors were screened for malaria using a malaria rapid diagnostic test (RDT) one week before they commenced collection and four weeks after the collection ended. Those who were malaria positive were treated per the national guidelines with an artemisinin-based combination therapy (ACT). All female *Anopheles* were preserved in labeled vials and transported to the laboratory for further processing. Identification of species, gender, and abdominal status was done in the field laboratory.

1.2.2 PYRETHRUM SPRAY CATCH (PSC)

The team conducted pyrethrum spray catches using a commercial aerosol containing 0.64 percent pyrethrum and 0.75 percent chlorpyrifos ethyl. VectorLink entomologists visited houses in the morning between 6 a.m. and 9 a.m. and laid white sheets on the floor and over furniture. All food, people, and animals were removed from the house and the windows and doors closed. A collector first sprayed along the eaves and any open space around the windows or gaps in the wall from the outside then proceeded inside and sprayed towards the walls and ceiling. After spraying, houses remained closed for 10-15 minutes. After this period, the sheets were retrieved from the house and examined for any mosquitoes that were knocked-down. Mosquitoes were subsequently sorted by genus as anopheline or culicine. All female *Anopheles* were further separated by abdominal status and categorized as unfed, blood-fed, half-gravid, or gravid. All female *Anopheles* were preserved in labeled vials and transported to the laboratory for further processing. Identification of species, gender, and abdominal status was done in the field laboratory. A total of twenty houses were sampled per month per site, totalling 160 houses per month across all sites. Mosquitoes were sampled from the same houses every month.

1.2.3 PARITY RATES

Female *Anopheles* 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 per site (or all collected if <50) collected from indoor and outdoor HLC was dissected and the ovaries observed to estimate the parity rate. Ovaries were dissected in the field, shortly after collection, 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 Health Sciences Research Institute / Institut de Recherche en Sciences de la Santé (IRSS) laboratory and stored at 4°C for further laboratory analysis.

1.2.4 RESIDUAL EFFICACY OF SUMISHIELD® 50WG AND ACTELLIC® 300CS

The team tested the residual efficacy of insecticide on the walls by conducting monthly cone bioassays from June to December 2018 in IRS sites. Cone bioassays were conducted to assess quality assurance of IRS 48 hours to 72 hours after spraying with Actellic 300CS (Kongoussi and part of Solenzo) or Sumishield 50WG (Kampti and part of Solenzo). The World Health Organizaition (WHO) protocol for the evaluation of residual efficacy (WHO, 2016) was performed using two to five-day-old unfed females of *An. gambiae* Kisumu, a reference strain that is susceptible to all insecticides, that is reared at the IRSS/Centre Muraz insectary. The team also collected wild *An. gambiae* s.l. larvae in each IRS site and reared them in the insectary to the adult stage, for monthly cone bioassay in parallel with the susceptible colony. A total of four treated houses (two houses made of cement and two made of mud) were randomly selected in each district for bioassay. In each house, six plastic cones were placed on three walls of the house, two cones per wall, one cone for *An. gambiae* Kisumu strain and one for wild *An. gambiae* s.l.. Two were placed at 1.5 m on one wall, two at 1m and two at 0.5 m on the other two. Ten mosquitoes were tested per cone. Mosquitoes were taken back for delayed mortality assessment after 24 hours at 80 ± 10 percent relative humidity and 27 ± 2°C temperature. Tests were performed monthly from T0 to T6 after treatment (June to December 2018).

The team also assessed the fumigant effect 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 fifty unfed female *An. gambiae* Kisumu and *An. gambiae* s.l. from each site. They were exposed in ten plastic tubes, with ten females tested per tube. Two were placed per house, with one for *An. gambiae* Kisumu strain and one for wild *An. gambiae* s.l.. 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.

1.2.5 INSECTICIDE SUSCEPTIBILITY TESTS

The team collected *An. gambiae* s.l. larvae from different larval habitats such as gutters, tires, shallow wells, and pools of standing water from each locality, brought them to the IRSS insectary and reared them to adults prior to use in bioassays. The purpose of this collection was to assess the insecticide resistance status of adult *An. gambiae* s.l. The team sampled at least 10 larval habitats per site and pooled them to obtain a representative sample size.

The team used WHO tube tests to monitor the susceptibility of wild *An. gambiae* populations to insecticides commonly used in public health and agriculture from the NMCP and PMI (sprayed and control) sentinel sites nationwide (Figure 2). Batches of twenty-five unfed, three to five-day-old adult females were exposed to filter papers impregnated with:

- 0.05% deltamethrin
- 4% PBO followed by 0.05% deltamethrin
- 0.75% permethrin
- 0.1% bendiocarb
- 0.25% pirimiphos-methyl
- 2% clothianidin (prepared by the project)

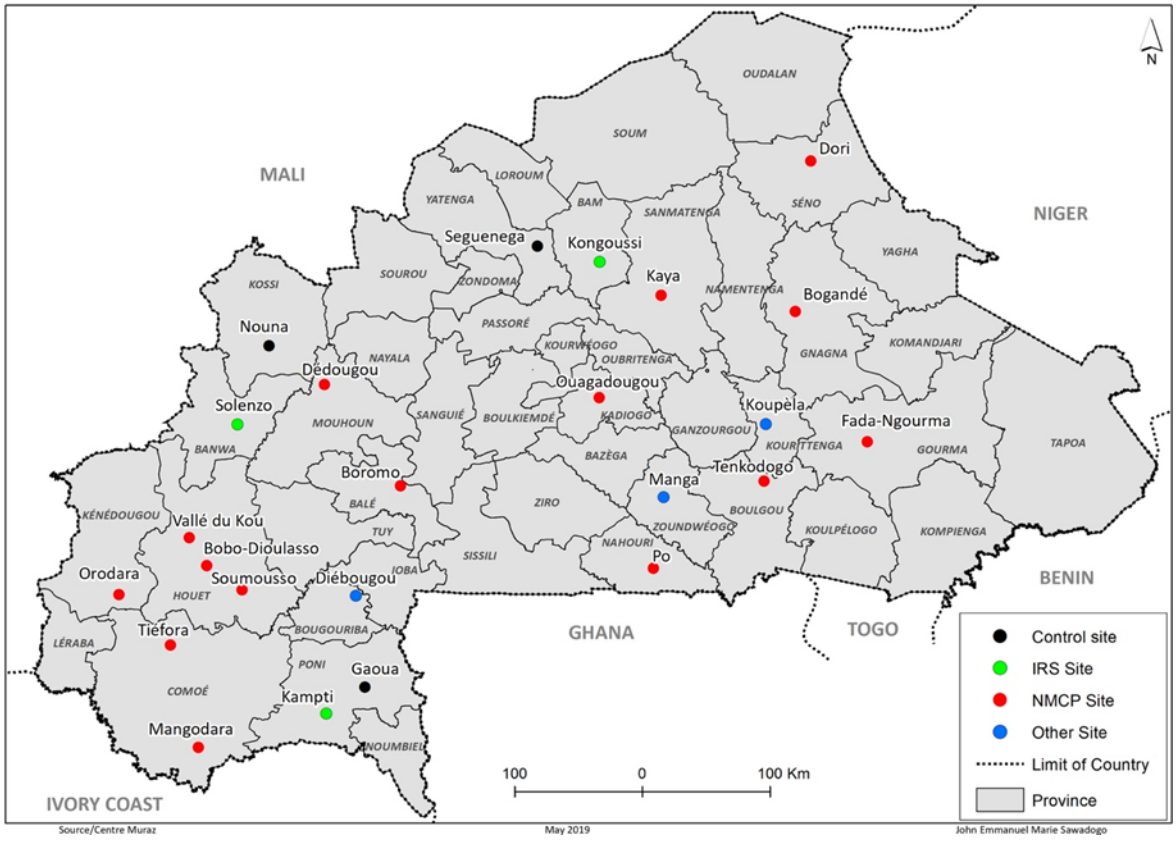
And bottle bioassay for one insecticide:

- 100µg/bottle chlorfenapyr (prepared by the project)

The team also conducted susceptibility tests with diagnostic dosages of new insecticides clothianidin and chlorfenapyr in the PMI longitudinal sites including IRS and their control sites (six sites) and

Mangodara, a recent PMI site added in 2017 (Figure 2). All filter papers were provided by WHO, except clothianidin papers that were prepared by IRSS. The team used the CDC bottle method to test chlorfenapyr at the interim diagnostic dose of 100µg/bottle. Other sites were also tested when mosquitoes were available. The team performed insecticide resistance intensity assays with deltamethrin using the CDC bottle technique which involved coating 250ml bottles with varying concentrations of deltamethrin at 1x (12.5µg), 2x (25µg), 5x (62.5µg) or 10x (125µg) the diagnostic concentration. The bottles were air dried overnight and two to five-day-old mosquitoes were exposed in the treated bottles with mortality recorded at the diagnostic time of 30 minutes. Resistance intensity tests were conducted in IRS sites and controls for a total of eight sites.

Figure 2: Map showing sites for insecticide resistance monitoring in 2018.



1.3 LABORATORY ANALYSES

1.3.1 ANOPHELES GAMBIAE S.L. PLASMODIUM FALCIPARUM INFECTION RATE

All mosquitoes dissected for their parity status in the field and stored in the lab at -20°C were processed by polymerase chain reaction (PCR) to determine infectivity rates with *P. falciparum*. The team used the heads and thorax of all female *Anopheles gambiae* s.l. specimens for the PCR analyses as described by Morassin et al. (2002) and adapted by Sangaré et al. (2013). The mosquitoes were also identified to species level by PCR (Santalomazza et al., 2008).

1.3.2 ORIGIN OF BLOOD MEAL (ANTHROPOPHILY RATE)

Blood-fed *An. gambiae* s.l. from PSC were used to assess host preference for blood meal source. A random selection of 50 specimens per site per month were tested by a direct enzyme-linked immunosorbent assay (ELISA) (Beier et al., 1988) using anti-host (IgG) conjugated against human, bovine, pig, donkey and sheep blood..

1.3.3 MOLECULAR SPECIES IDENTIFICATION OF THE *AN. GAMBIAE* COMPLEX AND FREQUENCY OF KDR L1014 F AND ACE-1R MUTATIONS

A subsample of 50 female *An. gambiae* s.l. were identified by PCR per insecticide, including 25 live and 25 dead. Genomic DNA of mosquitoes was extracted with 2 percent cetyl trimethyl ammonium bromide (2% 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 the *V*gsc-1014F West Africa *kdr* mutation was identified according to the protocol of Martinez-Torres et al. (1998) and the *V*gsc-1014S East Africa *kdr* mutation by the protocol described by Ranson et al. (2000). The *ace-1R* G119S mutation will be identified by PCR- RFLP as described by Weill et al. (2004).

1.4 DATA ANALYSIS

The human biting rate (HBR) was determined as the number of mosquitoes biting a person per night (indoor and outdoor) as determined from HLCs. The sporozoite infection rate (IR) was calculated as the proportion of mosquitoes (primarily *An. gambiae* s.l.) found to be positive for *P. falciparum* DNA in the head or thorax. The entomological inoculation rate (EIR) was defined as HBR 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 calculations were performed using R software, version 3.5.2. To analyze the variables of interest (mosquito density, IR and EIR), 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 nbinom1 or nbinom2. A difference is considered as significant when the p-value is less than 0.05.

2. RESULTS

2.1 VECTOR SPECIES COMPOSITION (JUNE TO DECEMBER 2018)

Between June and December 2018, a total of 32,263 culicidae were collected, including 18,728 anopheline mosquitoes. Other species including *Culex* (13,237), *Aedes* (209), and *Mansonia* (89) were collected by HLC (see annex, Table 1) and 2,843 *An. gambiae* s.l. were collected by PSC (see annex, Table 2). *An. gambiae* s.l. was the most abundant caught by HLC reaching 17,034 (91%), followed by *An. nili* with 1,546 (8.2%), with a small number of *An. funestus*, *An. coustani* and *An. pharoensis* consisted of 0.8% (Figure 3). The main malaria vectors in Burkina Faso are *An. gambiae* s.l. and *An. funestus*, with *An. nili*, *An. coustani*, and *An. pharoensis* possible minor vector species.
The abundance of *An. nili*, *An. funestus*, and *An. coustani* was greater in Gaoua and Kampti in the south west from July to October and very rare in northern areas.

Figure 3: *Anopheles* species composition based on HLCs for all sites combined (3 IRS sites + 3 unsprayed))

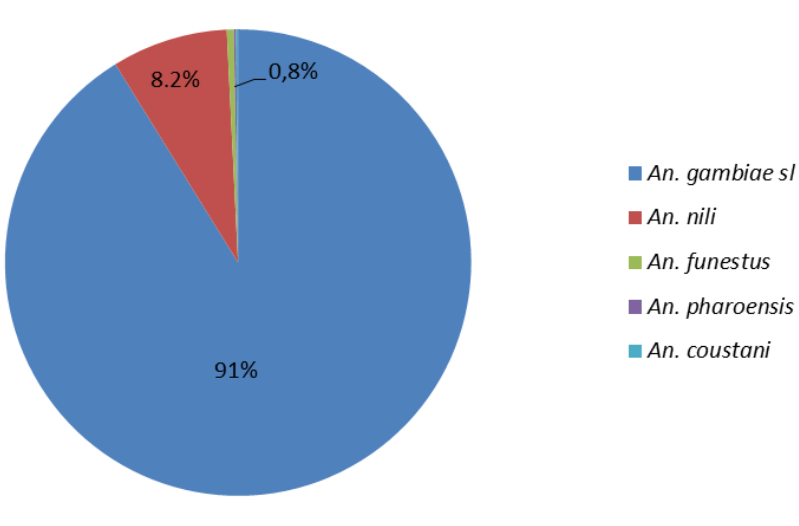


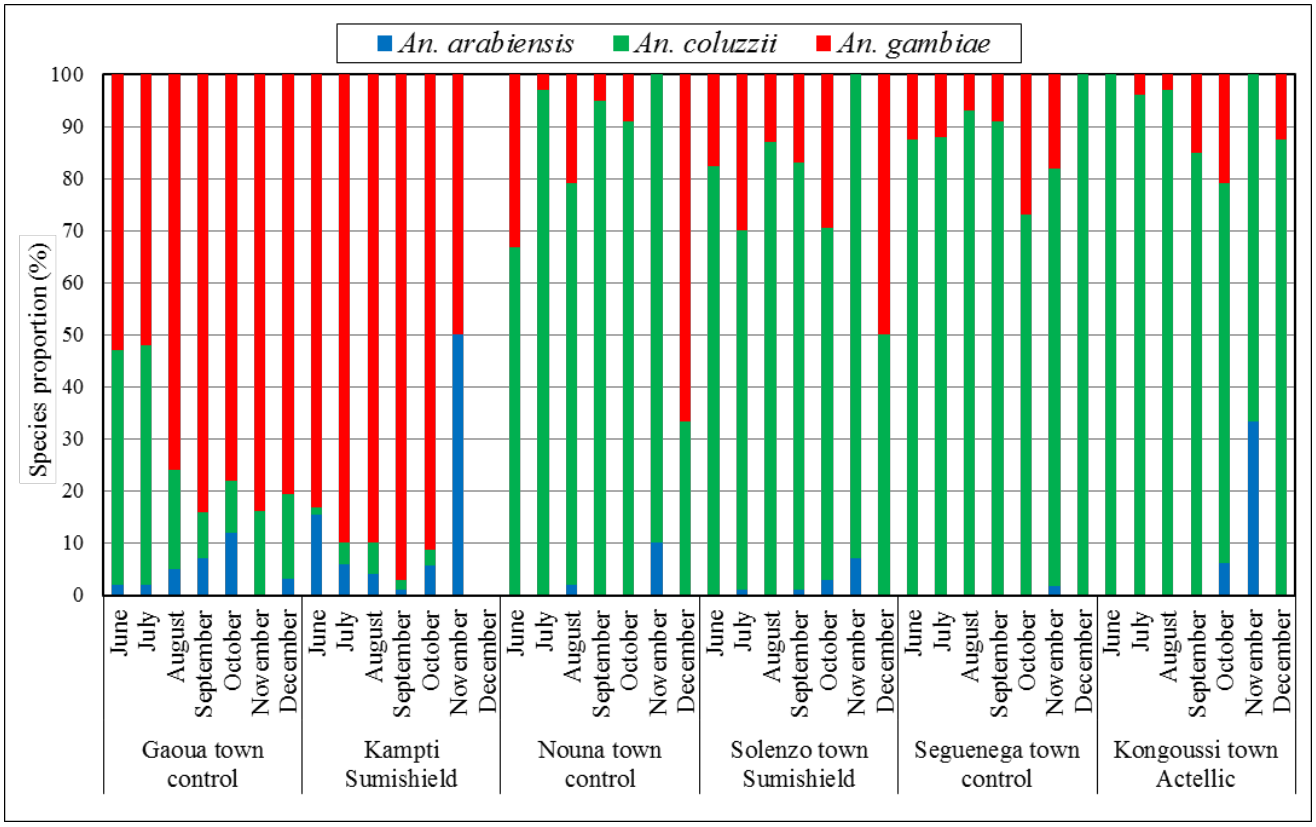
Figure 4 presents monthly molecular species data for *An. gambiae* s.l. collected by HLC at the six longitudinal monitoring sites. *An. coluzzii* was predominant in Nouna, Seguenega, Kongoussi, and Solenzo (see Figure 4). More than 80 percent of *Anopheles* in Kampti were *An. gambiae*, while Gaoua had relatively similar frequencies of *An. gambiae* and *An. coluzzii* in June and July, with other months dominated by *An. gambiae*.

An. arabiensis was found in low frequencies in Gaoua, Solenzo, and Kongoussi. The frequency of *An. arabiensis* increased in Kampti during November 2018, to be approximately equal to that of *An. gambiae* (Figure 4). No *An. gambiae* s.l. were collected by HLC or PSC in Kampti in December. In June, the number of mosquitoes collected was low and the samples tested by PCR were less than 100 (except in Gaoua).

It is interesting that the proportion of *An. arabiensis* being collected in the southwestern regions is increasing compared to previous years. However, in the central and northern areas, the proportion of *An. arabiensis* was very low in contrast to previous years.

In summary, *An. gambiae* was the predominant malaria vector species in the South West (Gaoua and Kampti), while *An. coluuzzi* was more frequent in the Centre North (Seguenega and Kongoussi) and Centre West (Nouna and Solenzo).

Figure 4: Species composition identified by PCR within the *An. gambiae* s.l. complex collected by HLC in all sites (n=100 per month/site, except in June).



2.2 MALARIA VECTOR BITING RATES (JUNE TO DECEMBER)

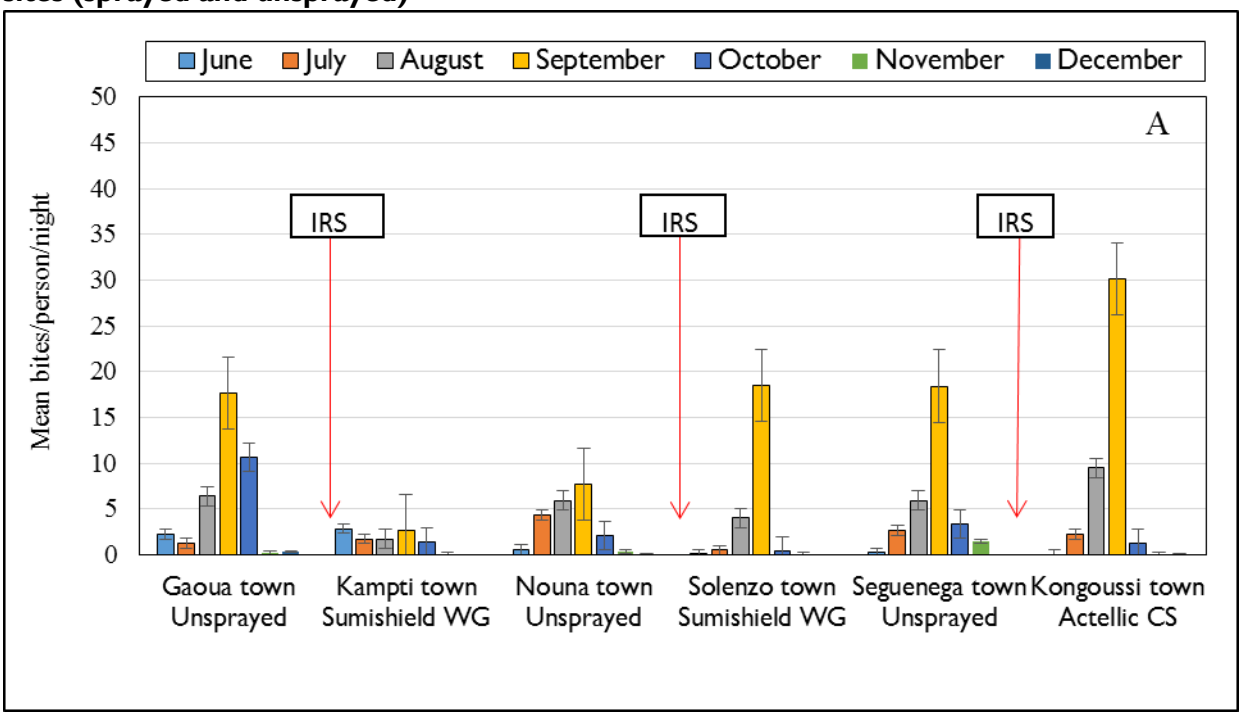
When analyzing the mean bites per person per month indoors during the period of June through December, the overall number of *An. gambiae* s.l. bites were three times lower in Kampti town (sprayed with SumiShield WG) compared to Gaoua town, its unsprayed control site (Figure 5). However, the biting rate in the rural Kampti site, which was also sprayed with SumiShield WG, was extremely high, with the mean indoor biting rate greater than twenty bites per person per night for four months (Figure 5B).

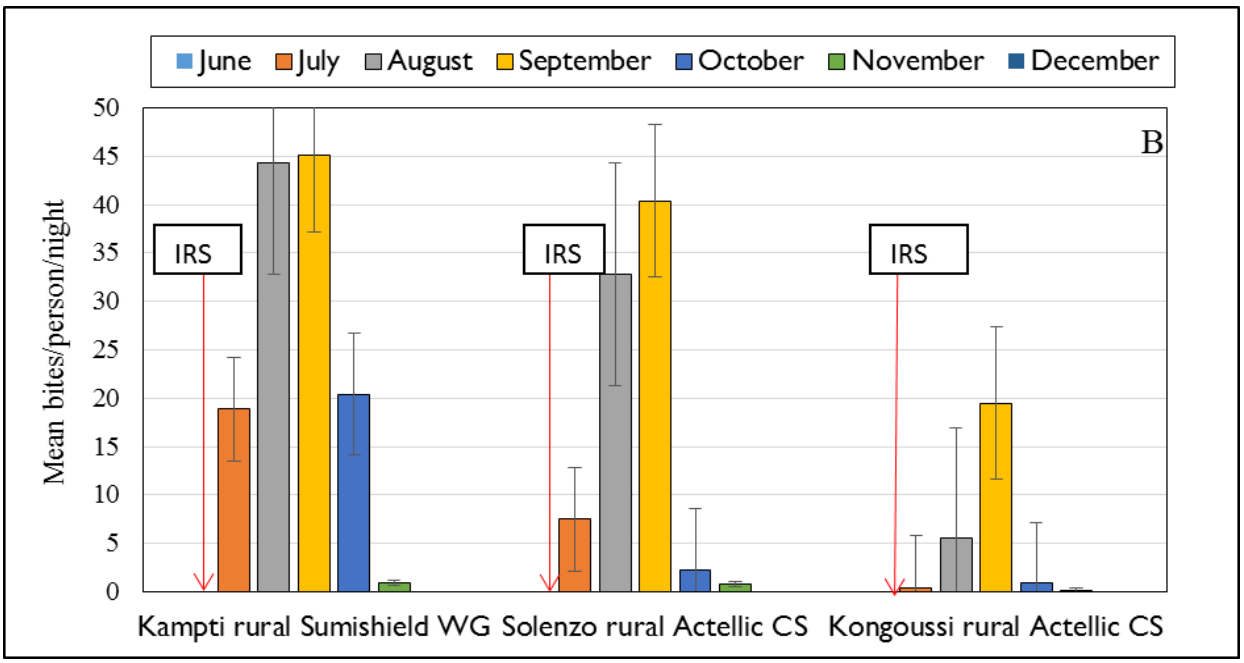
Indoor *An. gambiae* s.l. biting rates were also generally lower in Solenzo town (sprayed with SumiShield WG) than neighboring unsprayed Nouna town, except for a large biting peak in September in Solenzo, which reached close to 20 bites per person per night (Figure 5A). In 2017, there was also a large biting peak in September 2017 in Solenzo, where Actellic CS was sprayed. The biting rates in rural Solenzo (sprayed with Actellic CS) (Figure 5B) were particularly high when compared to the semi-urban town site of Solenzo (Figure 5A). In Kongoussi town (sprayed with Actellic CS), the biting rates did not differ

from unsprayed Seguenega town, especially during peak biting in September, when biting rates were higher in Kongoussi and reached 30 bites per person per night.

Indoor biting rates were much higher in sprayed rural sites (Figure 5B) than semi-urban sites (Figure 5A), except Kongoussi rural site which had similar results. Unfortunately, there were no unsprayed rural sites monitored for direct comparison. Peak biting rates were in September for most sites, with particularly high biting peaks in rural sites, reaching up to 45 bites per person per night in rural Kampti, despite being sprayed with SumiShield WG (Figure 5B).

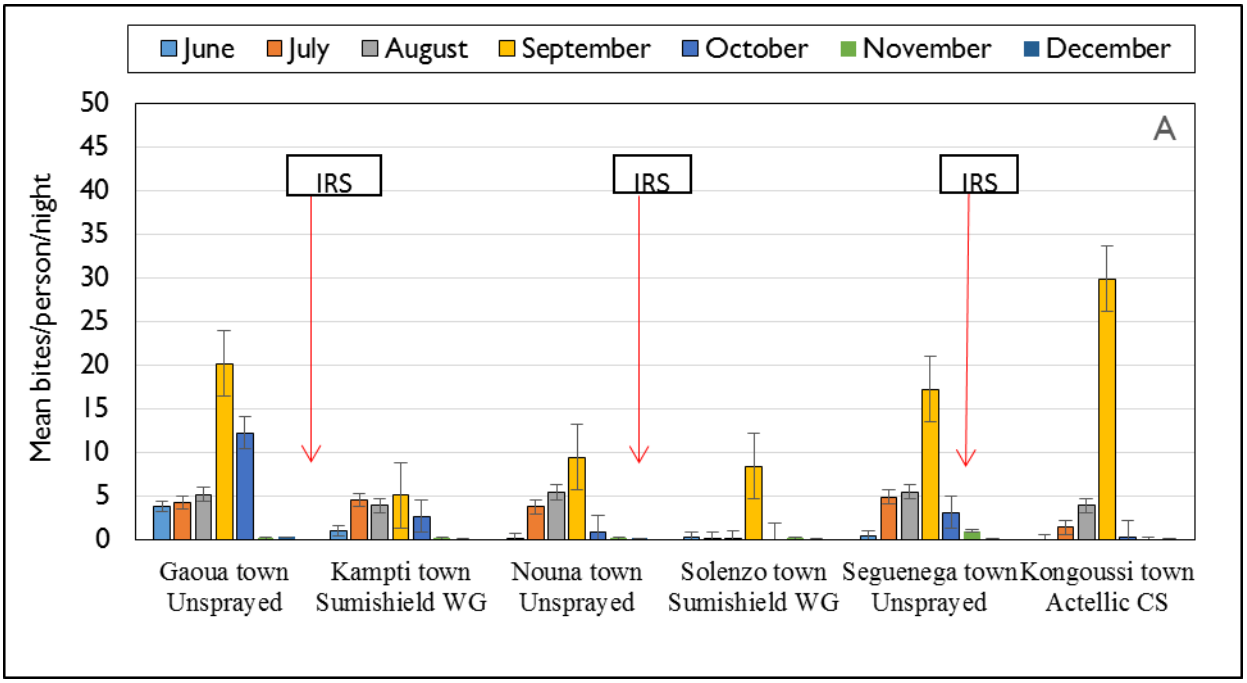
Figure 5A: Mean An. gambiae s.l. bites/person/night from indoor HLC in semi-urban town sites (sprayed and unsprayed)

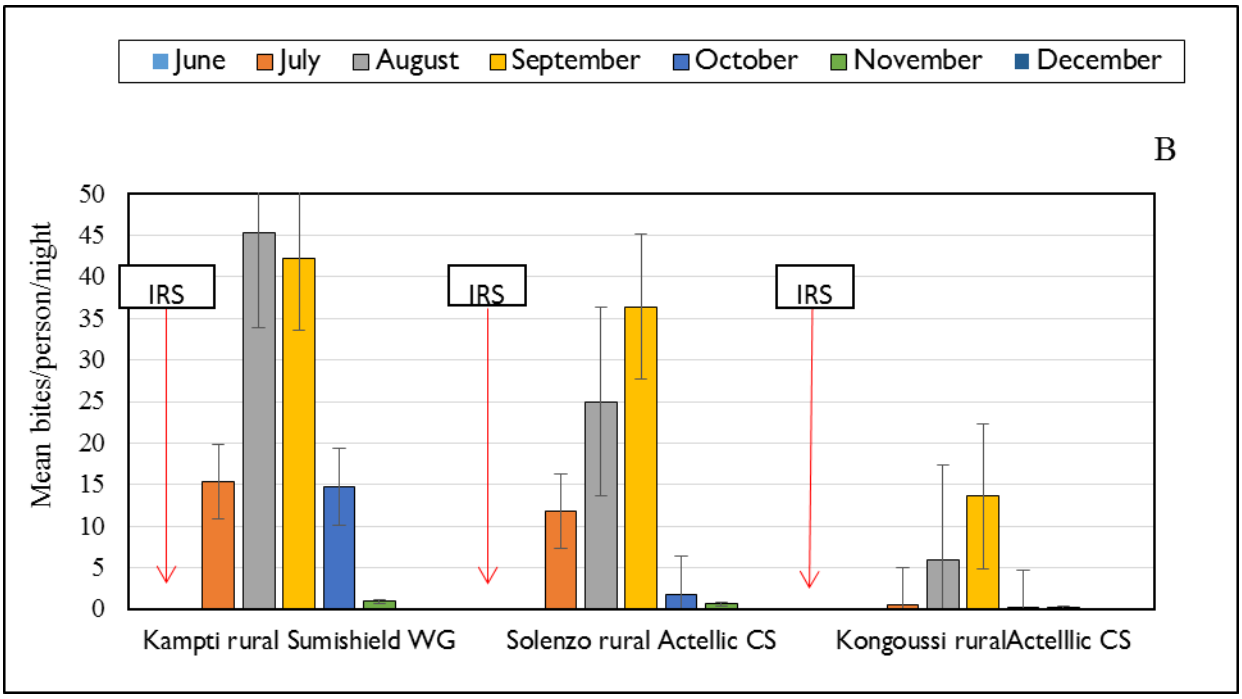




The outdoor biting rates in semi-urban town sites were lower in the sprayed sites of Kampti and Solenzo compared to their control (unsprayed) sites in Gaoua and Nouna towns (Figure 6A). However, in the sprayed site of Kongoussi town (Actellic CS), there was a large outdoor biting peak in September, reaching close to 30 bites per person per night (Figure 6A). In the rural sprayed sites of Kampti (Sumishield WG) and Solenzo (Actellic CS), outdoor biting rates were particularly high (>25 bites per person per night in August through September) and comparable to indoor biting rates. Even without unsprayed rural sites for comparison, these high biting rates in sprayed sites are concerning.

Figure 6: Mean *An. gambiae* s.l. bites/person/night from outdoor HLC in A) semi-urban town sites (sprayed and unsprayed) and B) in rural sites (sprayed)



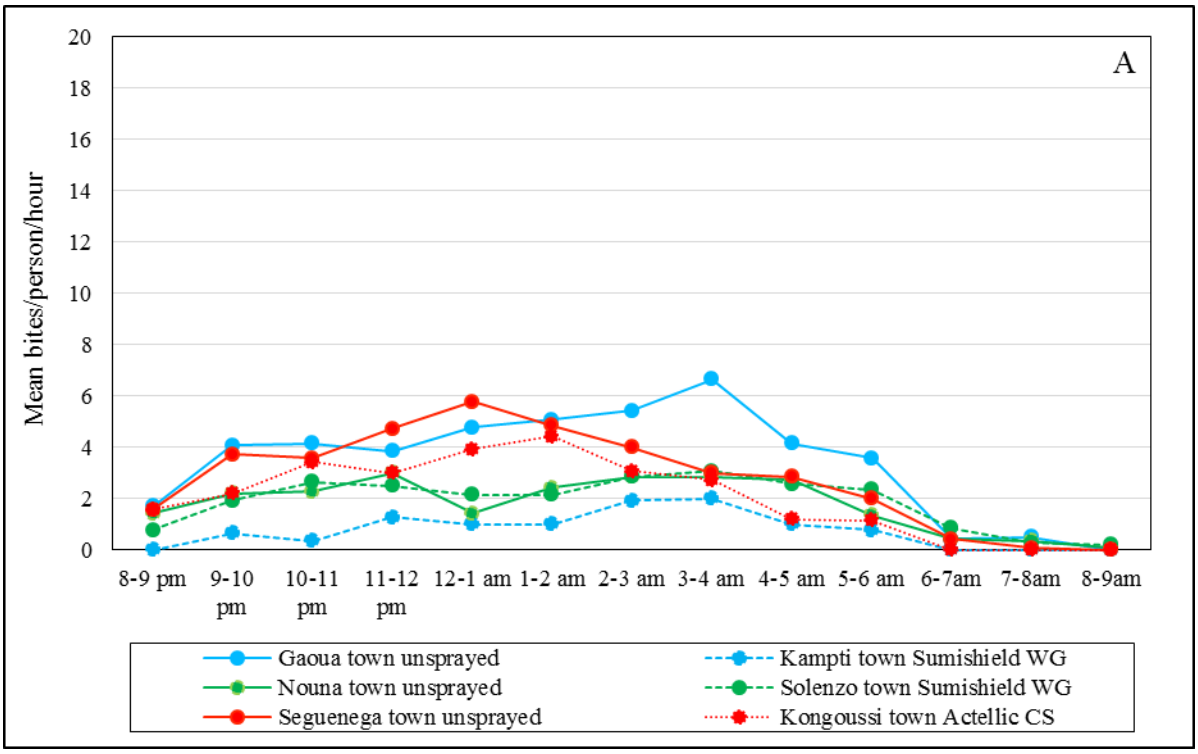


2.3 BITING TIMES OF *AN. GAMBIAE* S.L.

The predominant malaria vector species were *An. gambiae* in Kampti and Gaoua, with *An. coluzzii* the most common in the remaining north-western and northern sites (see section 3.1). Indoor biting of *An. gambiae* s.l. started at 8:00 p.m. (when monitoring began), and gradually increased up to midnight, with the biting peak between midnight and 5:00 a.m. in most sites (Figure 7A). The indoor *An. gambiae* s.l. biting peak was not very pronounced, with continuous biting in most sites between 10 p.m. and 5 a.m. There were no clear differences in the time of biting in sprayed and unsprayed sites. In the Solenzo rural site (sprayed with Actellic CS), there was some evidence of an earlier indoor biting peak, between 9 p.m. and midnight (Figure 7B) and a later indoor peak in Kampti.

Outdoor biting timing was broadly similar to indoor trends (Figure 8A). There were few pronounced biting peaks, except in Nouna and Gaoua where biting peaks were recorded between 2 a.m. and 6 a.m. Despite variation by site, biting intensities were generally higher between midnight and 5 a.m.There was no clear difference in biting times between sprayed and unsprayed sites.

Figure 7: Biting times of *An. gambiae* s.l. bites/person/hour from indoor HLC collection in A) town sites (sprayed and unsprayed) and B) rural sites (sprayed) from June to December 2018



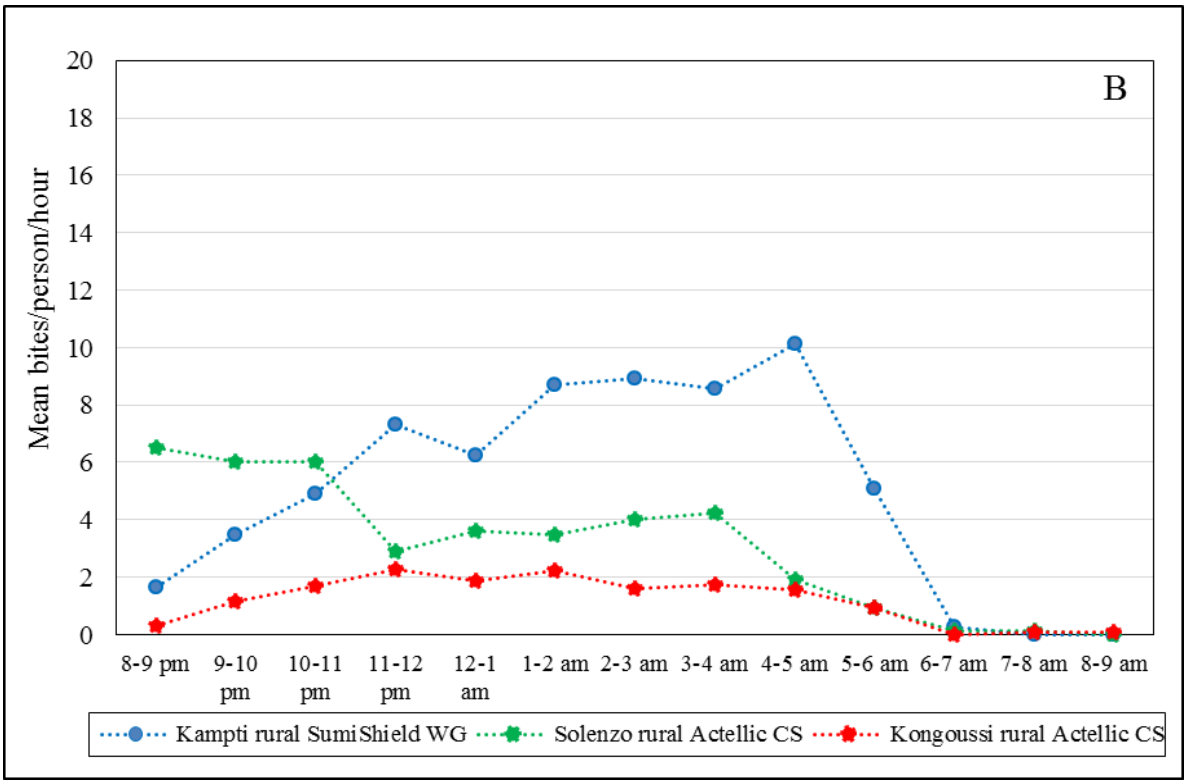
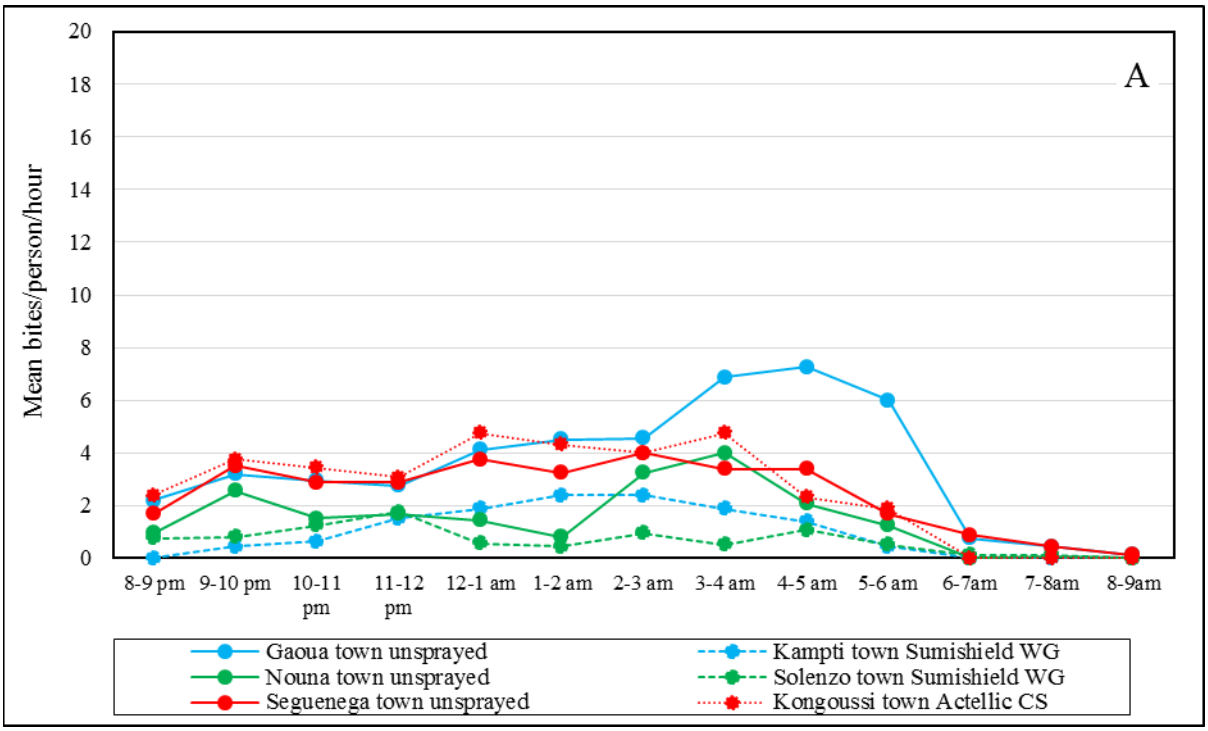
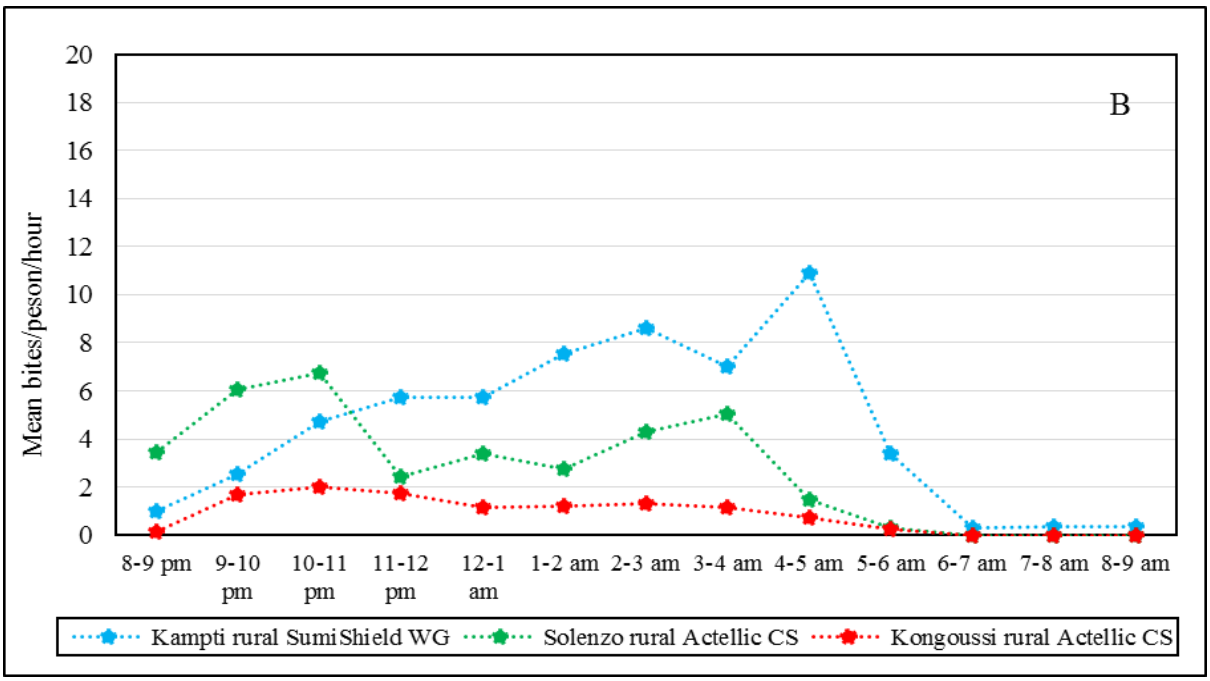


Figure 8: Biting times of *An. gambiae* s.l. bites/person/hour from outdoor HLC collection in A) central sites (sprayed and unsprayed) and B) rural sites (sprayed) from June to December 2018.





2.4 MALARIA VECTOR INDOOR RESTING RATES (JUNE TO DECEMBER)

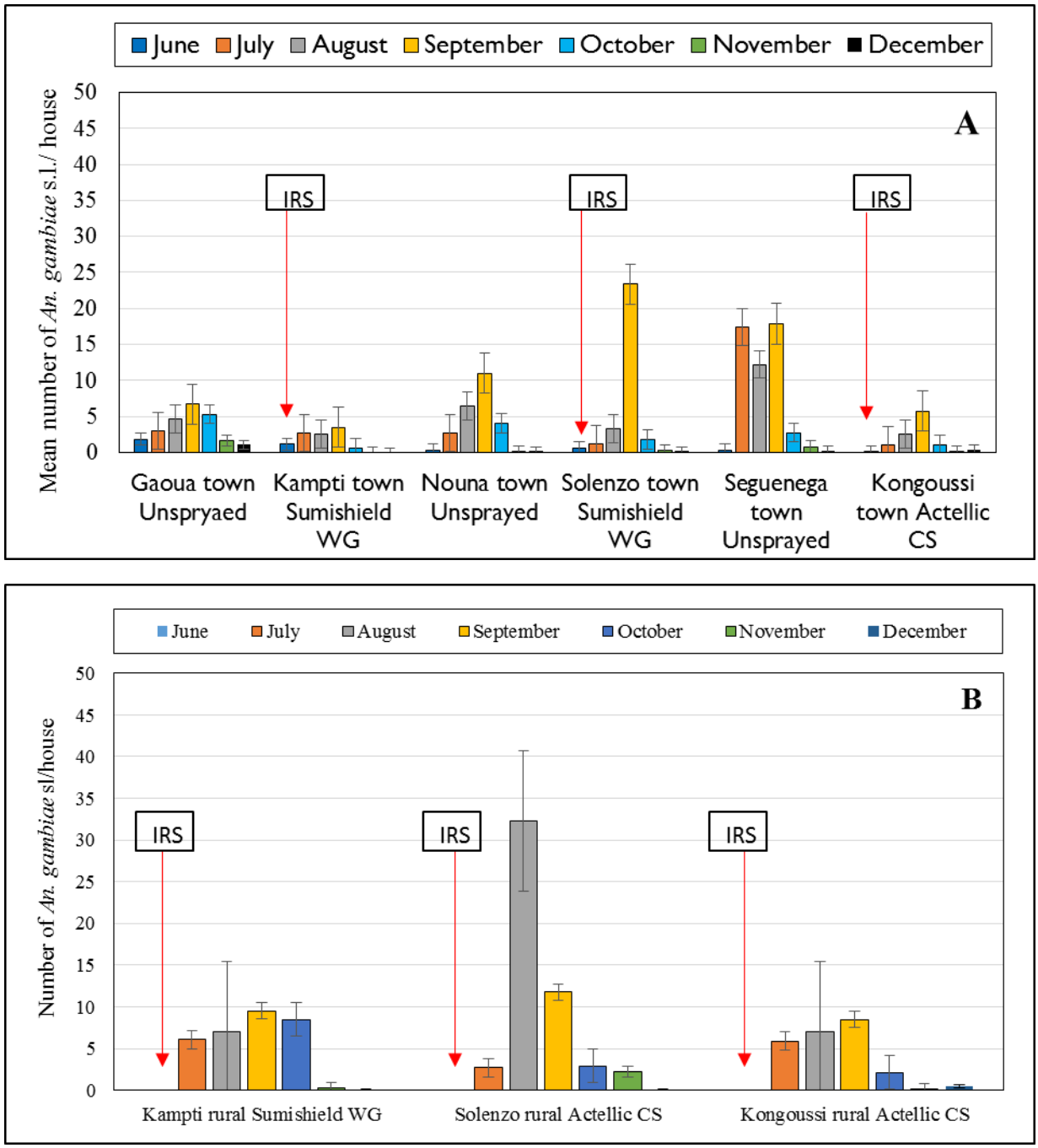
An. gambiae s.l. indoor resting densities were lower in the sprayed site of Kampti town than the neighboring unsprayed site of Gaoua (Figure 9A). Indoor resting densities were lower in Kongoussi town compared to Seguenega town, but not in Solenzo compared to Nouna (Table 1). In Solenzo town (SumiShield WG) a particularly large indoor resting density was recorded in September (Figure 9A). In the sprayed sites of Kampti (Sumishield WG) and Kongoussi (Actellic CS), indoor resting densities were below six *An. gambiae* s.l. per house per day throughout the monitoring period. Indoor resting densities were high from July to September in the unsprayed site of Seguenega with 10-20 *An. gambiae* s.l recorded per house per day (Figure 9A). In rural sprayed sites, the indoor resting densities were relatively low when compared to the high biting rates. The monthly indoor resting density was generally <10 *An. gambiae* s.l. per house per day, except in Solenzo in August when a particularly high indoor resting density was recorded (Figure 9B).

Table 1: Comparison of post-spray average density per house of *An. gambiae* s.l. from pyrethrum spray catch between IRS sites and their paired control sites (unsprayed) in town sites.

Collection method	Category	Mean	RR (95% CI)
Pyrethrum Spray Catch (PSC)	Gaoua town unsprayed	3.44	1
	Kampti town Sumishield WG	1.01	0.24 (0.07-0.76) *
	Nouna town unsprayed	3.52	1
	Solenzo town Sumishield WG	4.06	0.73 (0.34-1.55)
	Seguenega town unsprayed	7.36	1
	Kongoussi town Actellic CS	1.31	0.20 (0.08-0.47) ***

Legend: *P*= 0.0001'***'; *P*=0.001'**'; *P*=0.01'*'

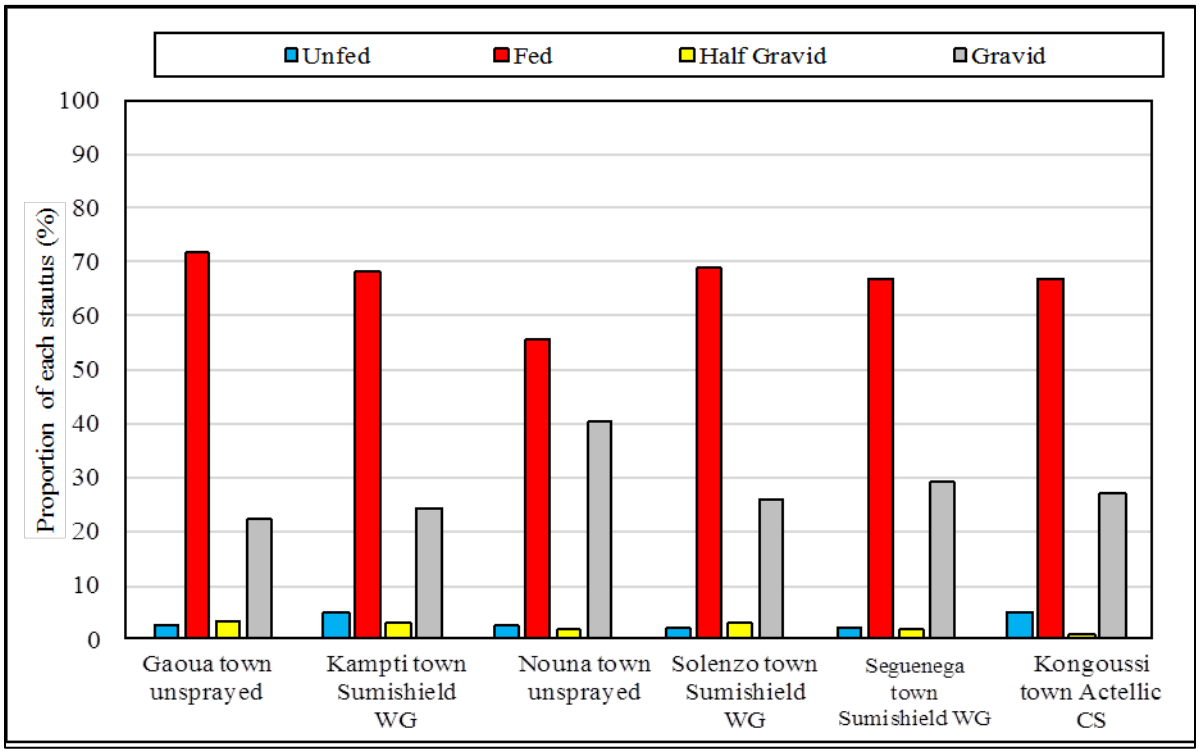
Figure 9: Mean number *An. gambiae* s.l. /house from indoor PSC in A) semi-urban town sites (sprayed and unsprayed) and B) in sprayed rural sites.

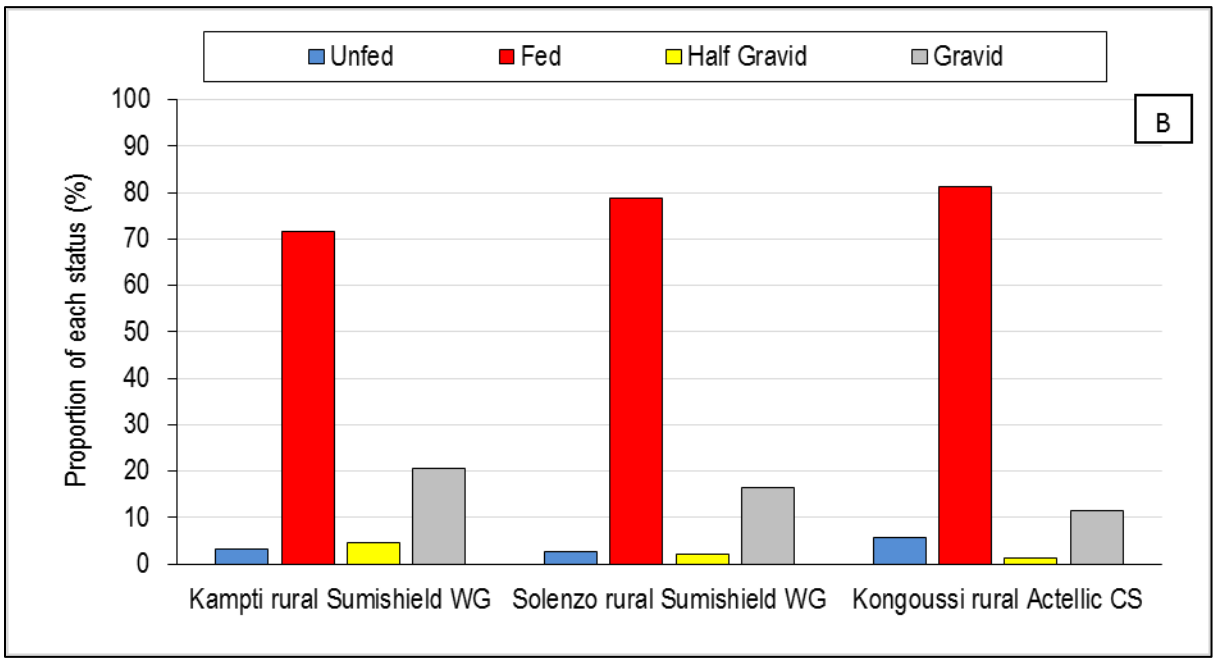


2.5 BLOOD MEAL SOURCE OF *AN. GAMBIAE* S.L. COLLECTED BY PSC

In all sites, the majority of females collected early in the morning were freshly blood-fed and the proportion of gravid females ranged from 22 percent to 40 percent (Figure 10). The proportion of unfed and half gravid *An. gambiae* s.l. was <5 percent.

Figure 10: Physiological status of *An. gambiae* s.l. collected by PSC among all districts A) in town sites and B) rural sites.



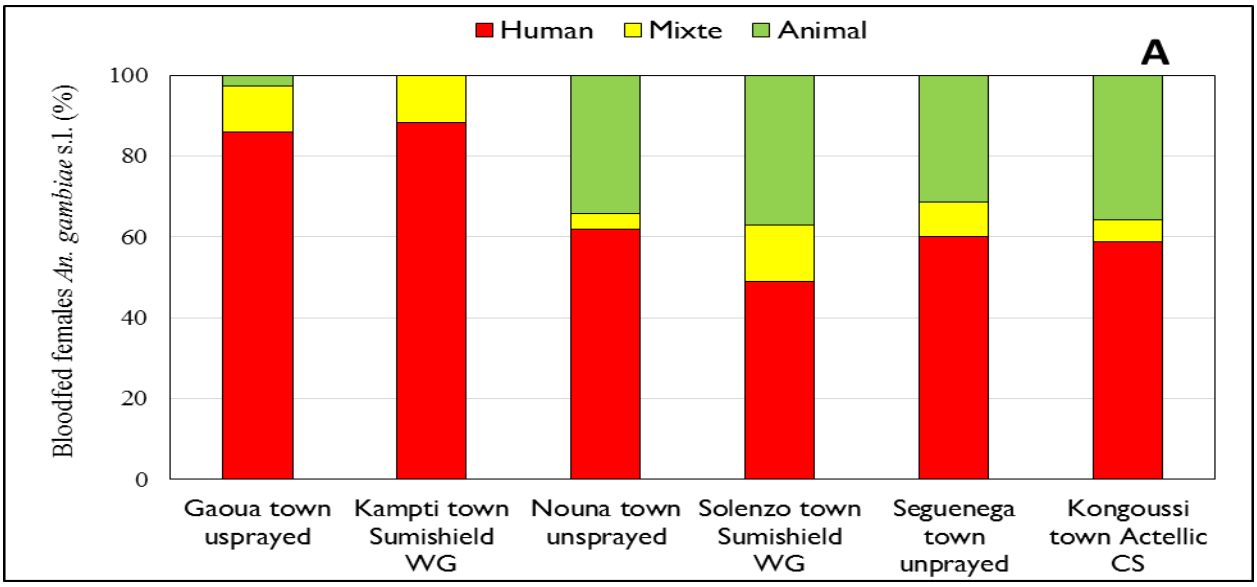


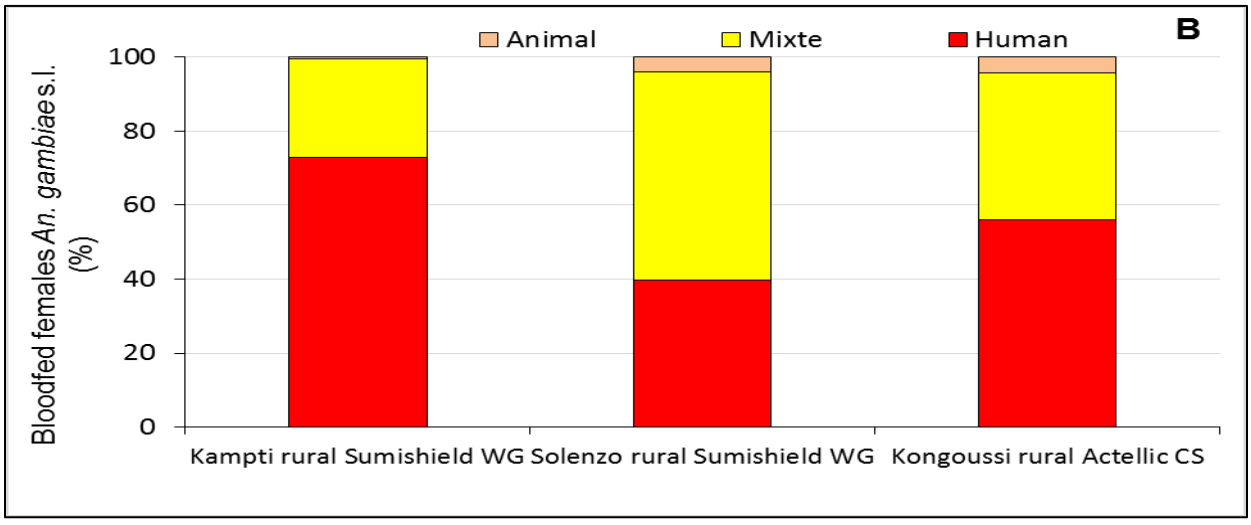
More than 1,100 abdomens of blood-fed *An. gambiae* s.l. were individually processed and analyzed to determine the blood-meal source. *An. gambiae* s.l. were particularly anthropophagic in Gaoua and Kampti, with approximately 85 percent of blood meals originating from humans and another 10 to 15 percent from mixed human and animal. In the Central and Northern sites, there was a greater proportion of animal feeding, with only approximately 50 to 65 percent of blood meals originating from humans. An estimated 2 to 13 percent of *An. gambiae* s.l. took mixed human/animal blood meals.

The most common *An. gambiae* s.l. blood meals from animals, in order of frequency, were goat/sheep followed by pig and cattle (Annex 3). In the central and northern sites, a large proportion of mixed blood meals was found to be mostly more than one type of animal blood but there were also human and animal blood meals in Gaoua and Solenzo. In conclusion, *An. gambiae* s.l. was extremely anthropophilic in the south-west regions like Gaoua and Kampti, and relatively more plastic in the North.

Overall, *An. gambiae* s.l. was extremely anthropophagic in the South West sites and more zoophilic in the Central and Northern sites (Figure 11).

Figure 11: Blood meal source of *An gambiae* s.l. collected by PSC from town sites (A) and rural sites (B)





2.6 MALARIA VECTOR PARITY RATES (JUNE TO DECEMBER)

Overall, parity rates were significantly lower in Kongoussi town (sprayed with Actellic CS) than in its unsprayed control Seguenega town ($\chi 2=8.63$, ddl 1, P<0,001) (Table 2). However, there were no differences between Kampti sprayed with SumiShield WG and Gaoua unsprayed town sites for parity rates. Similarly, in rural sprayed sites, the parity rates were similar to those found in town areas. Overall, there was no evidence that SumiShield WG had any impact on *An. gambiae* s.l. age structure. The parity rates from three rural sprayed sites were around 60-70 percent in the three sites of Kampti, Solenzo, and Kongoussi (Table 2).

Table 2: Parity rate of *Anopheles gambiae* s.l. females from semi-urban and rural sites (indoors & outdoors), June-December 2018

Sites	Total dissected	Parous	Non-parous	Parity rate (%)
Gaoua town unsprayed	448	334	114	74.6
Kampti town Sumishield WG	152	125	27	82.2
Nouna town unsprayed	307	221	86	72.0
Solenzo town Sumishield WG	81	61	20	75.3
Seguenega town unsprayed	424	278	146	65.6
Kongoussi town Actellic CS	180	95	85	52.8
Kampti rural Sumishield WG	372	252	120	67.7
Solenzo rural Actellic CS	236	163	73	69.1
Kongoussi rural Actellic CS	140	87	53	62.1

Table 3: Parity rate of *Anopheles gambiae* s.l. females from semi-urban sites (indoors & outdoors), June-December 2017.

Sites	Total dissected	Parous	Non-parous	Parity rate (%)
Gaoua town unsprayed	689	536	153	77.79
Kampti town Sumishield WG	377	321	56	85.15
Nouna town unsprayed	452	299	153	66.15
Solenzo town Sumishield WG	376	265	111	70.48
Seguenega town unsprayed	404	258	146	63.86
Kongoussi town Actellic CS	422	349	73	82.70

2.7 PLASMODIUM FALCIPARUM INFECTION RATES OF AN. GAMBIAE S.L.

The overall *P. falciparum* infection rates in *An. gambiae* s.l. were highest in the unsprayed southern site of Gaoua, town reaching 13 percent (Figure 12; Table 4) compared to the other sites ($\chi 2=6.82$, $P<0.01$).

The sporozoite rate was significantly lower indoors in Kampti town (sprayed with Sumishield WG) than in Gaoua town, the respective control site ($\chi 2=6.59$, $P=0.01$). The sporozoite rate appeared to be lower in sprayed sites in Solenzo and Kongoussi compared to their control sites (Nouna and Seguenega), but there was no significant difference. In summary, the infection rates were significantly lower only in Kampti (spray area) compared to its control site, Gaoua. Infected mosquitoes were only *An. gambiae* in Kampti and in Gaoua, whereas in the other sites it was supported by *An. coluzzii* (Figure 13A). The same tendancy was observed outdoors (Figure 13B). *An arabiensis* had played a small role only in Gaoua indoor and in Nouna outdoors.

Figure 12: Overall proportion (%) of *Anopheles gambiae* s.l. with *P. falciparum* DNA detected in head/thorax, June-December 2018 per site

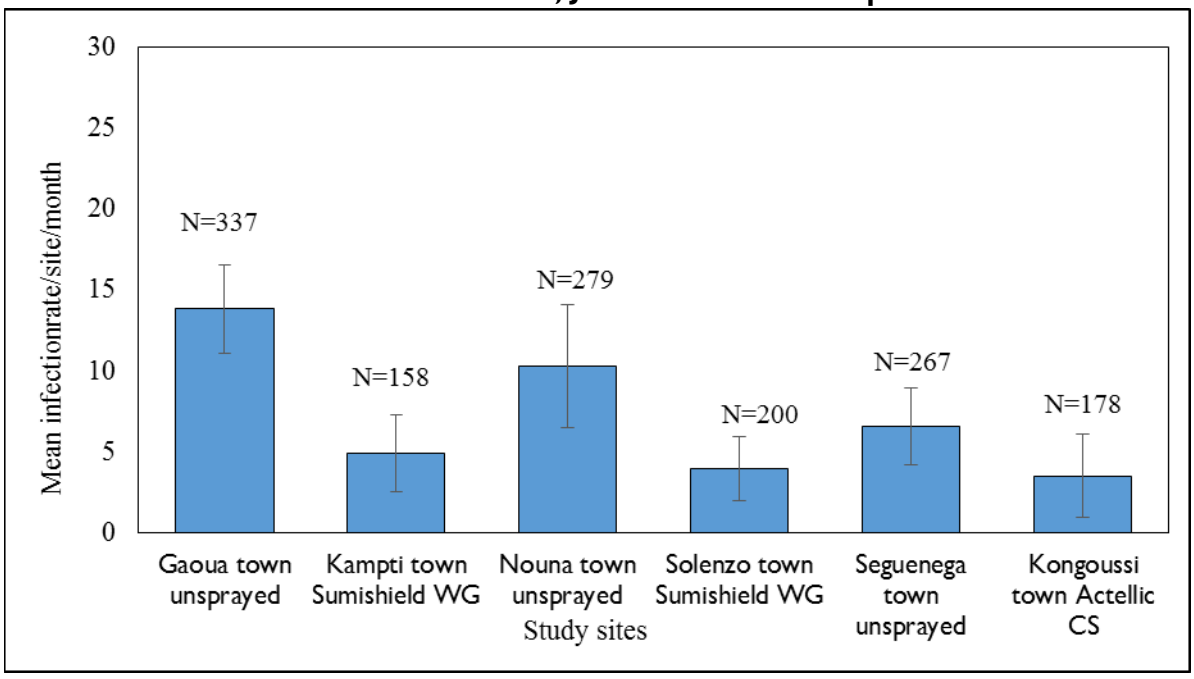
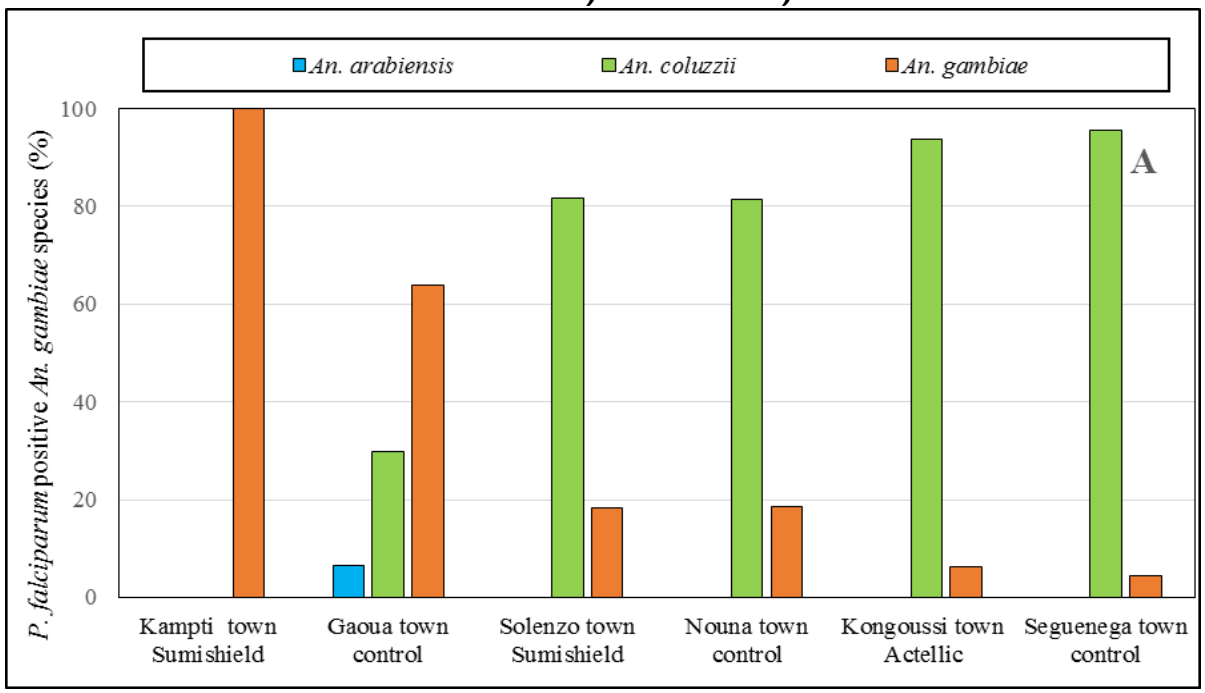


Figure 13: Proportion (%) of *P. falciparum* positive samples by species; *An. gambiae*, *An. coluzzii* and *An. arabiensis* from A) indoor and B) outdoor collections



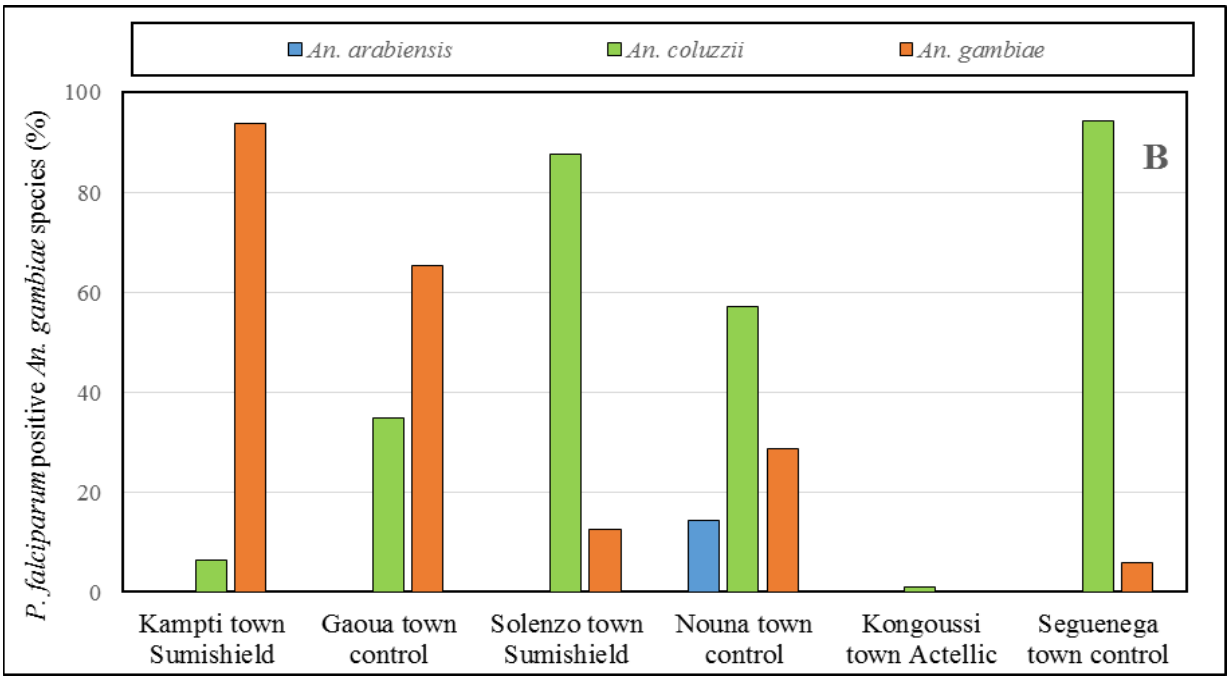


Table 4: Infection rates of An. gambiae s.l. from June to December 2018 in the IRS and control town sites

Sporozoite rate of *An. gambiae* s.l. from HLC indoor collections						
Months	**Gaoua town** unsprayed % (+/nb tested)	**Kampti town** Sumishield WG % (+/nb tested)	**Nouna town** unsprayed % (+/nb tested)	**Solenzo town** Sumishield WG % (+/nb tested)	**Seguenega town unsprayed** % (+/nb tested)	**Kongoussi town** Actellic CS % (+/nb tested)
June	17.24 (10/58)	3.84 (2/52)	0 (0/16)	7.69 (1/13)	0 (0/10)	0 (0/1)
July	26,22 (16/61)	0 (0/14)	1.69 (1/59)	7.69 (1/13)	16.27 (7/43)	0 (0/22)
August	10.41 (5/48)	13.33 (2/15)	13.11 (8/61)	0 (0/38)	7.84 (4/51)	1.78 (1/56)
September	7.69 (4/52)	6.12 (3/49)	2.22 (1/45)	5.08 (3/59)	5.55 (3/54)	18.18 (8/44)
October	18.18(10/55)	11.11(3/27)	15.11(13/86)	8.06(5/62)	6.15(4/65)	4.54(2/44)
November	13.88(5/36)	100 *(1/1)*	40(4/10)	0(0/13)	10.25(4/39)	0(0/3)
December	3.70(1/27)	0	0(0/2)	0(0/2)	0(0/5)	0(0/8)
Overall	**15.13% (51/337)**	**6.96% (11/158)**	**9.67% (27/279)**	**5% (10/200)**	**8.23% (22/267)**	**6.17% (11/178)**
June	11.90 (5/42)	7.69 (1/13)	0 (0/2)	0 (0/4)	0 (0/6)	0 (0/0)
July	5.12 (2/39)	0 (0/8)	2.43 (1/41)	(0/0)	5.26 (3/57)	0 (0/7)
August	11.53 (6/52)	20.68 (6/29)	5.12 (2/39)	13.33 (2/15)	8.16 (4/49)	6.81 (3/44)
September	12.5 (6/48)	0 (0/51)	3.63 (2/55)	20.5 (2/41)	15.21 (7/46)	1.78 (1/56)
October	8.88(4/45)	7.14(3/42)	14.28(2/14)	16.66(1/6)	11.42(4/35)	0(0/4)
November	0(0/1)	0(0/1)	0	0(0/1)	0(0/16)	0
December	0(0/4)	0	0(0/1)	0	0	0
Overall	**9.95% (23/231)**	**6.94% (10/144)**	**4.60% (7/152)**	**7.46% (5/67)**	**9.32% (18/193)**	**3.60% (4/111)**
Overall sporozoite rate (indoors +outdoors)						
Overall indoor + outdoor	**13.02% (74/568)**	**6.95% (21/302)**	**7.88% (34/431)**	**5.61% (15/267)**	**8.40% (40/476)**	**5.19% (15/289)**

Table 5: Infection rates of *An. gambiae* s.l. from June to December 2018 in the IRS and control rural sites

Sporozoite rate of *An. gambiae* s.l. from HLC indoor collections			
Months	**Kampti rural (SumiShield WG)** % (+/nb tested)	**Solenzo rural (Actellic CS)** % (+/nb tested)	**Kongoussi rural (Actellic CS)** % (+/nb tested)
June	0 (0)	0 (0)	0 (0)
July	17.39 (8/46)	8.6 (5/58)	12.24 (6/49)
August	19.04 (8/42)	5 (2/40)	0 (0/10)
September	16.92 (11/65)	1.81 (1/55)	7.57 (5/66)
October	11.11 (3/27)	8.06 (5/62)	4.54 (2/44)
November	1(1/1)	0(0/13)	0(0/3)
December	0	0(0/2)	0(0/8)
Overall	**17.13% (31/181)**	**5.65% (13/230)**	**7.22% (13/180)**
June	0 (0)	0 (0)	0 (0/0)
July	3.12 (1/32)	0 (0/29)	9.09 (2/22)
August	12.5 (5/40)	0 (0/40)	0 (0/4)
September	8.57 (3/35)	0 (0/45)	0 (0/34)
October	7.14 (3/42)	16.66 (1/6)	0(0/4)
November	0(0/1)	0(0/1)	0(0/0)
December	0	0	0
Overall	**8% (12/150)**	**0.8% (1/121)**	**3.125% (2/64)**
Overall sporozoite rate (indoors +outdoors)			
Overall indoor + outdoor	**8.15% (27/331)**	**6.45% (8/267)**	**10.345% (13/185)**

2.8 ENTOMOLOGICAL INOCULATION RATE

The EIR recorded in Solenzo town sprayed with Sumishield WG was also lower indoors compared to Nouna town unsprayed but there was no statistical difference (RR=0.49, IC95%= [0.14–1.75], *P*=0.27). The same pattern was observed outdoors (RR=0.72, IC95%= [0.23–2.28], *P*=0.58). Comparing the overall EIR (indoors and outdoors) of Solenzo town sprayed with Sumishield to Nouna unsprayed it was quite similar without any significant impact (RR=0.56, IC95%= [0.23–1.33], *P*=0.19). Similarly, comparing to 2017, it did not differ significantly (RR=3.31, IC95%= [0.80–13.57], *P*=0.09). However, if no significant reduction was recorded in Solenzo, it seemed that the EIR of Nouna increased in 2018 compared to 2017 (RR=4.19, IC95%= [2.13–8.24], *P*<0.001).

In Kongoussi town sprayed with Actellic CS the EIR recorded indoors were significantly lower with 51 infected bites and did not differ to its control unsprayed site, Seguenega town with 68 infected bites (RR=0.78, IC95%= [0.23–2.65], *P*=0.70). The outdoors EIR were significantly lower than in Seguenega (RR=0.17, IC95%= [0.05–0.56], *P*=0.003). The overall EIR (indoors and outdoors) were significantly reduced in Kongoussi town vs Seguenega unsprayed town (RR=0.17, IC95%= [0.05–0.56], *P*=0.003). Compared to 2017, the overall EIR in Kongoussi was reduced in 2018 (RR=0.27, IC95%= [0.08–0.84], *P*=0.024) whilst in Seguenega it increased a lot (RR=11.65, IC95%= [3.71–36.55], *P*<0.001).

It was noted that overall in 2018 malaria transmission increased, probably due to an intensive rainy season particularly in the Centre and North, when compared to 2017. In Kampti the impact of IRS was greater than the other sites. Overall, the impact of IRS was more prominent in areas with intensive

malaria transmission such as the South West followed by the Centre and moderately in Kongoussi vs Seguenega where the intensity of malaria transmission is short (only between August and October). Furthermore, September remained a key month in malaria transmission in this northern area where the same intensity was noted as last year. The intensity of this EIR was due to *An. gambiae* in the West and to *An. coluzzii* in the Centre-North as they were the dominant species respectively.

When following the monthly dynamic, the EIR estimated indoors indicated that transmission occurred at least 5 months in unsprayed areas from July to October in South West and delayed from August to October in the centre and centre North (Fig 14A). There was also outdoor transmission risk through October in the unsprayed and IRS areas (Fig 14B). The peak was observed in September from Solenzo to Kongoussi indoors but in August in Nouna and delayed to October in Gaoua averaging 50 i/b/p/m (Figure 14A).
In the sprayed sites the EIR indoors was drastically reduced in Kampti compared to its control site in Gaoua (all month long) (Anova test, F=3,056, R=0,19, *P*<0,05) and also in the two other sites except in September. Indeed, the EIR in September in Solenzo and Kongoussi were the highest, peaking at 44 i/b/p/m in Kougoussi followed by Gaoua in October. The outdoors EIR trends were similar but the peak was observed in September with the maximum in Gaoua and Seguenega with 78 i/b/p/m.

The EIR recorded in rural sprayed sites were more intensive both indoors and outdoors than those of any control town sites (Figures 15A &B).

Figure 14: Monthly Infected bites recorded indoors A) and outdoors B) in town sites from IRS sprayed villages and their respective control ones.

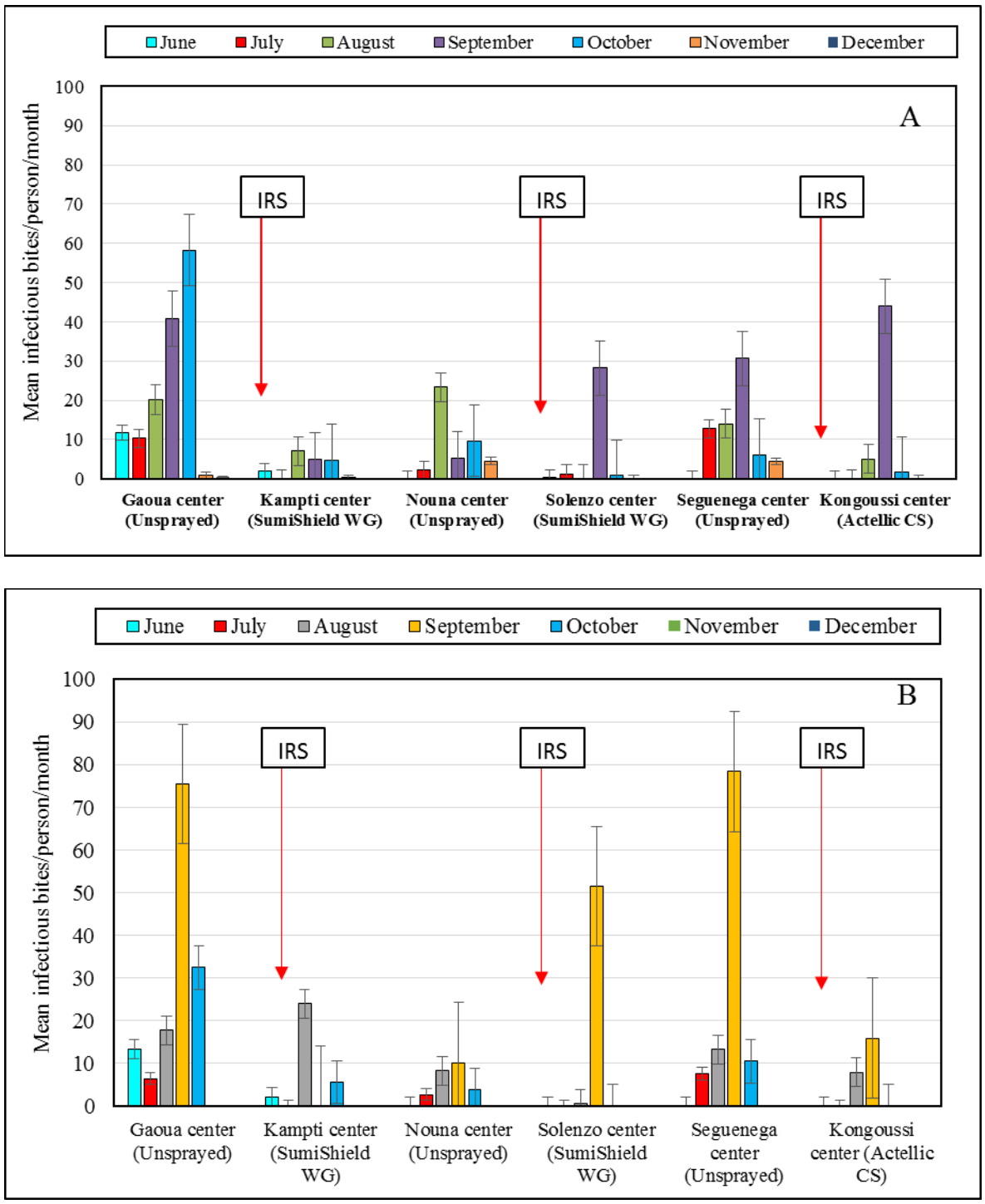
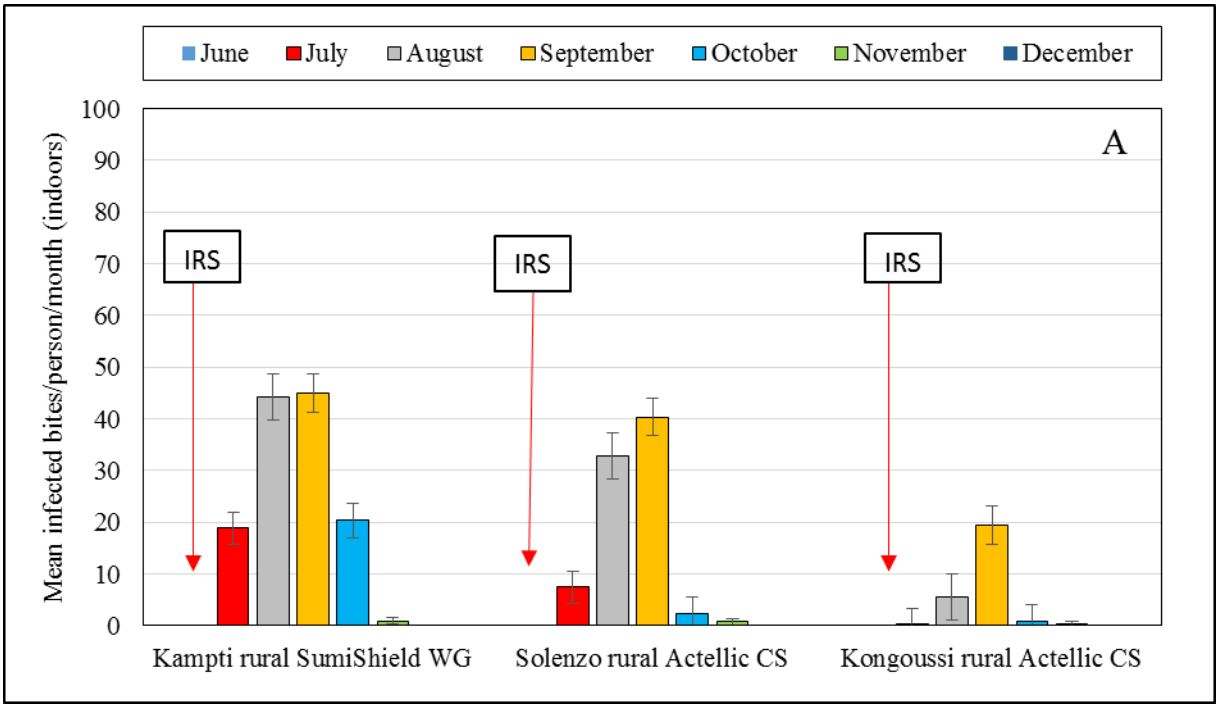


Figure 15: Monthly infected bites recorded A) indoors and B) outdoors in rural sites from IRS sprayed villages.



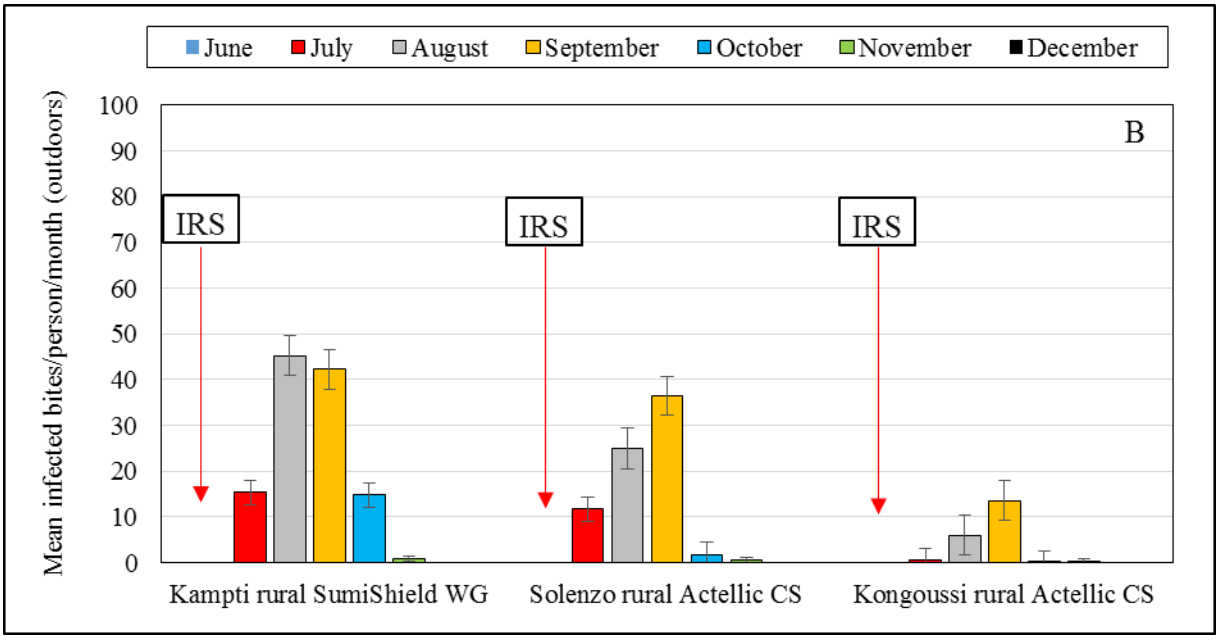


Table 6: Seven-month entomological inoculation rate (EIR) of *An. gambiae* s.l. and *An. nili* from indoors and outdoors catches in IRS and unsprayed control towns sites from June to December 2018.

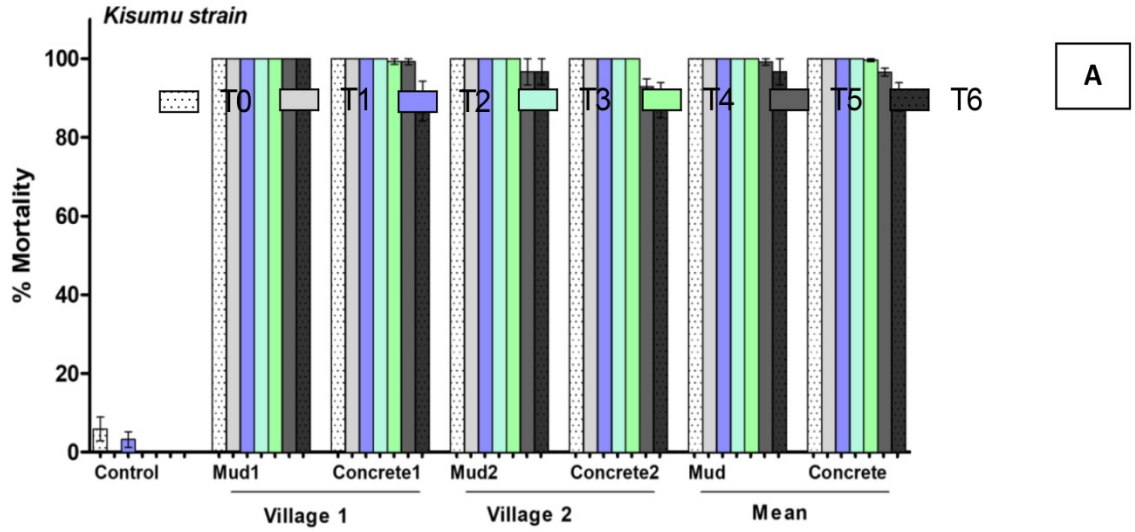
Treatment	Species	Indoors	Outdoors	Total
Gaoua town unsprayed	*An. gambiae* s.l.	142	145	293
	An. nili	5	1	
Kampti town Sumishield WG	*An. gambiae* s.l.	19	32	51
	An. nili	0	0	
Nouna town unsprayed	*An. gambiae* s.l.	45	25	70
Solenzo town Sumishield WG	*An. gambiae* s.l.	31	52	83
Seguenega town unsprayed	*An. gambiae* s.l.	68	110	178
Kongoussi town Actellic CS	*An. gambiae* s.l.	51	24	75

2.9 RESIDUAL EFFICACY OF SUMISHIELD® 50WG AND ACTELLIC® 300CS AGAINST SUSCEPTIBLE STRAIN *AN. GAMBIAE* "KISUMU" AND WILD *AN. GAMBIAE* S.L.

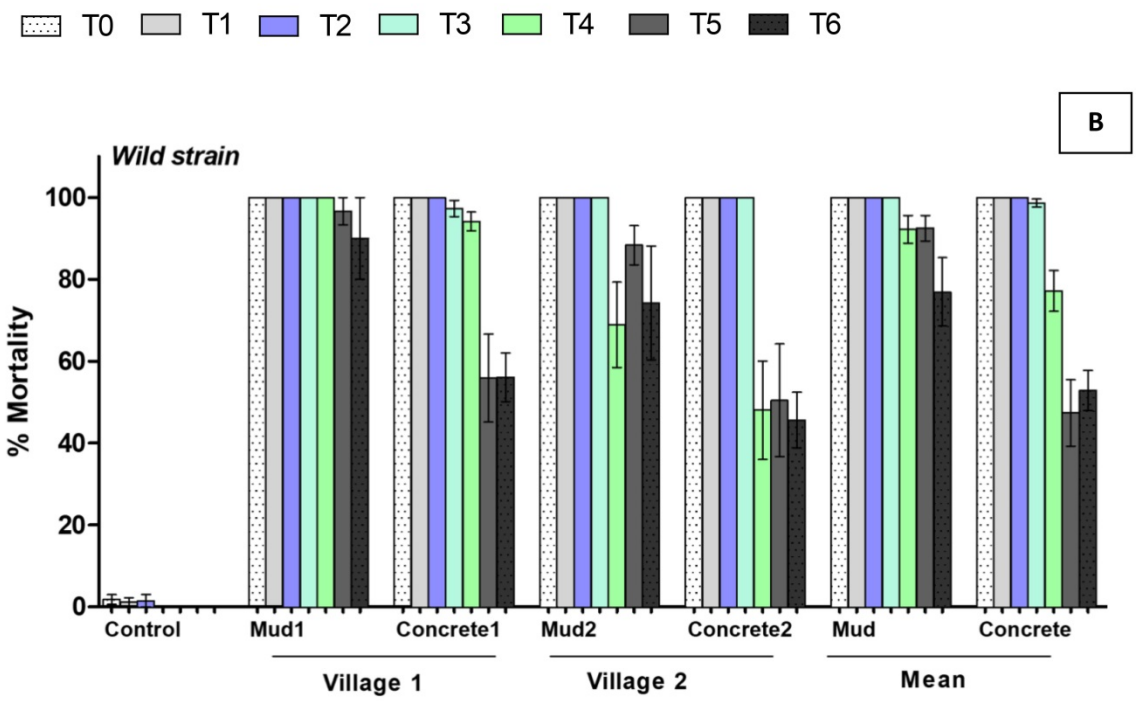
Monthly cone bioassays were conducted from June to December 2018 to determine the residual efficacy of each insecticide (SumiShield® 50WG and Actellic® 300CS) in terms of mortality, using the susceptible *An. gambiae* Kisumu insectary colony and wild *An. gambiae* s.l. mosquitoes. In Kongoussi, the mortality rate of *An. gambiae* Kisumu was 98 to100 percent for the first four months after which it declined to 90-95 percent, particularly on concrete walls at T5 and T6 (Figure 16A). The tests performed with wild *An. gambiae* s.l. also indicated high mortality rates during the first four months, but declined to 50 to 56 percent on concrete walls in the two villages (urban and rural sites) five and six months after spraying. There was also reduced mortality on mud walls in the second village between four and six months after spraying to between 70 and 95 percent (Figure16B). The mortality observed on the control unsprayed wall was lower than 5 percent at all time periods. Cone bioassay tests will continue in 2019 until mortality is less than 80 percent for two consecutive months on all substrates.

Figure 16. Mortality Rate (24h) of *Anopheles gambiae* "Kisumu" and wild *Anopheles gambiae* s.l using monthly WHO cone bioassay of sprayed walls in Kongoussi (Actellic® 300CS).

T0 =June (48h after spraying) T1 =July T2 = August T3 = September T4 =October T5 = November T6 = December

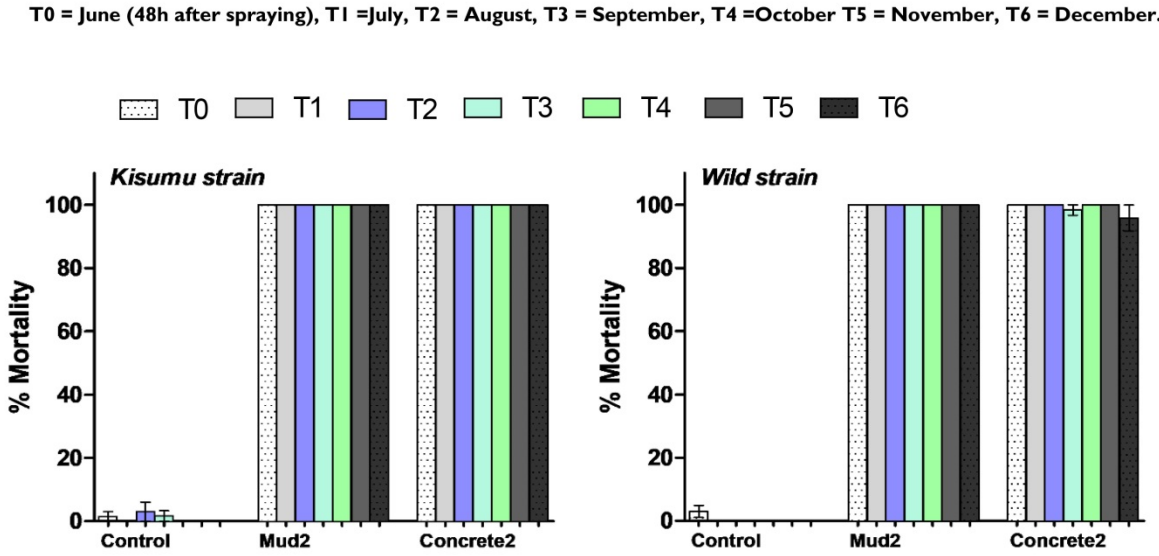


T0 = June (48h after spraying), T1 =July, T2 = August, T3 = September, T4 =October T5 = November, T6 = December



In Solenzo, houses were sprayed with Actellic® 300CS in a rural area (Molé). The mortality rates reached 100 percent even after six months with walls made with mud and cement both for Kisumu and >95 percent for wild *An. gambiae* s.l. (Figure 18). The control mortality was <2 percent for all tests.

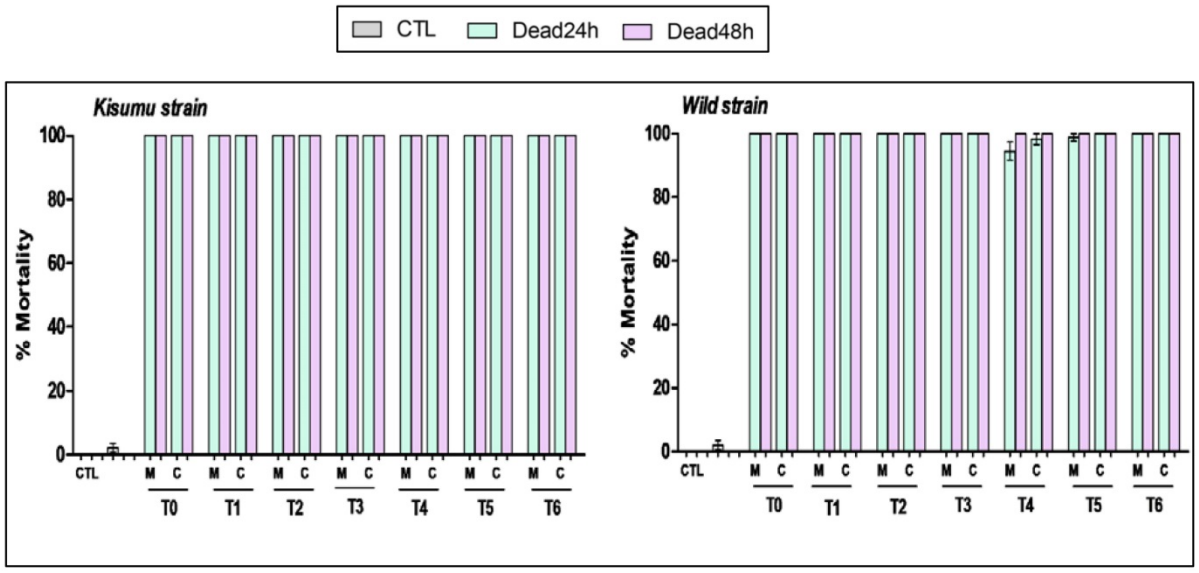
Figure 17. Mortality Rate (24h) of *Anopheles gambiae* "Kisumu" and wild *Anopheles gambiae* s.l. using monthly WHO test cones in Molé, Solenzo district (Actellic 300 CS).



Sumishield ® 50WG, sprayed in the central part of Solenzo, resulted in 100 percent mortality of both Kisumu and wild *An. gambiae* s.l. (within 48h of exposure) on both mud and cement walls six months after spraying. A slight reduction was noted with *An. gambiae* s.l. four months after spraying, but full efficacy was recorded 48 hours after exposure (Figure 18).

Figure 18: Mortality Rate (24h and 48h for SumiShield) of *Anopheles gambiae* Kisumu and wild *Anopheles gambiae* s.l. using WHO test cones in Solenzo district (center sprayed with SumiShield® 50WG).

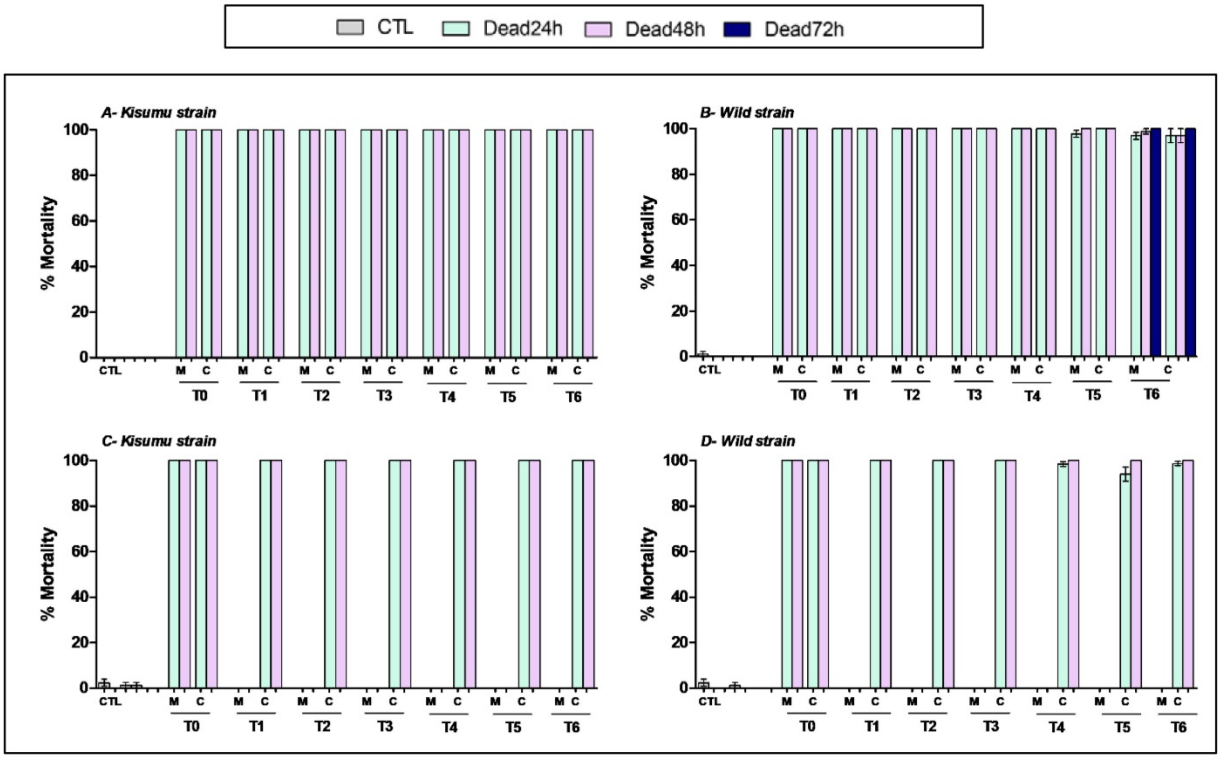
T0 = June (48h after spraying), T1 =July, T2 = August, T3 = September, T4 =October sT5 = November, T6 = December. CTL: Control, M: Mud wall and C: Concrete wall



In Kampti, cone bioassay was conducted in both town and rural district sites. In the rural site of Loglona (Kampti district), both mud and cement walls were found and tested in bioassays. In central Kampti, nearly all houses were made of cement. During the six-month evaluation (June to December), the efficacy of SumiShield® 50WG always reached 100 percent with "Kisumu" in Loglona and Kampti center (Figure 19A and C). With wild *An. gambiae* s.l., mortality was slightly delayed, but 100 percent mortality occurred within 72 hours of exposure (Figure 19B and D).

Figure 19: Mortality Rate (24h, 48h, 72h for SumiShield® 50WG) of *An. gambiae* Kisumu and wild *An. gambiae* s.l. rural site of Loglona (A and B) and Kampti center (C and D).

CTL : control, M : mud wall and C : concrete wall.

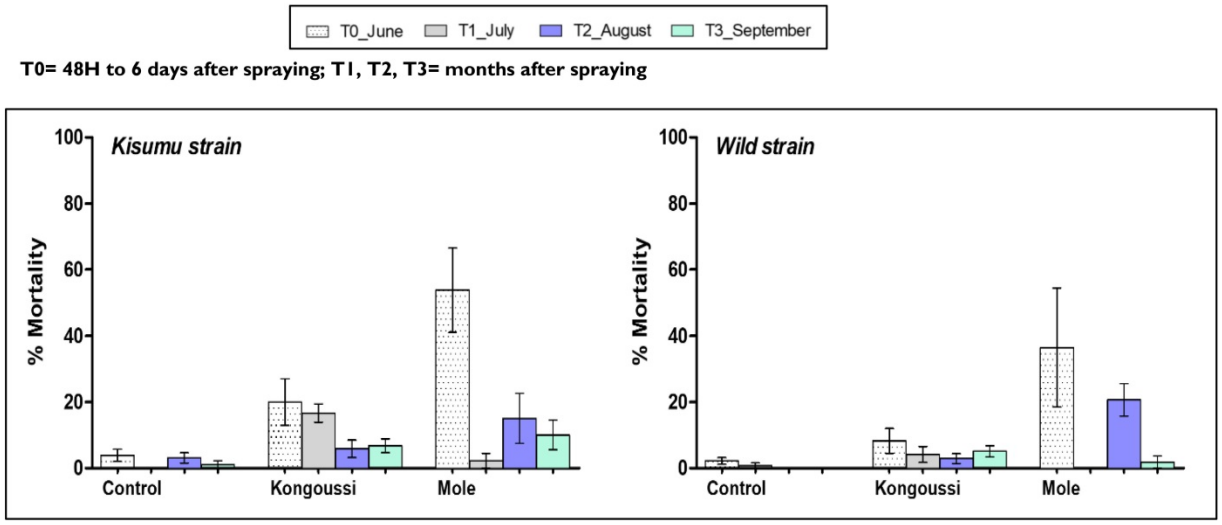


In all sites, Sumishield WG showed efficacy of at least six months on cement and mud walls with both insectary and wild *An. gambiae* s.l.; while Actellic CS had a residual efficacy of at least six months with the susceptible strain but only four months in Kongoussi with wild *An. gambiae* s.l. This result could be an indication of pirimiphos-methyl resistance in *An. gambiae* s.l. from Kongoussi.

Airborne Fumigant Mortality

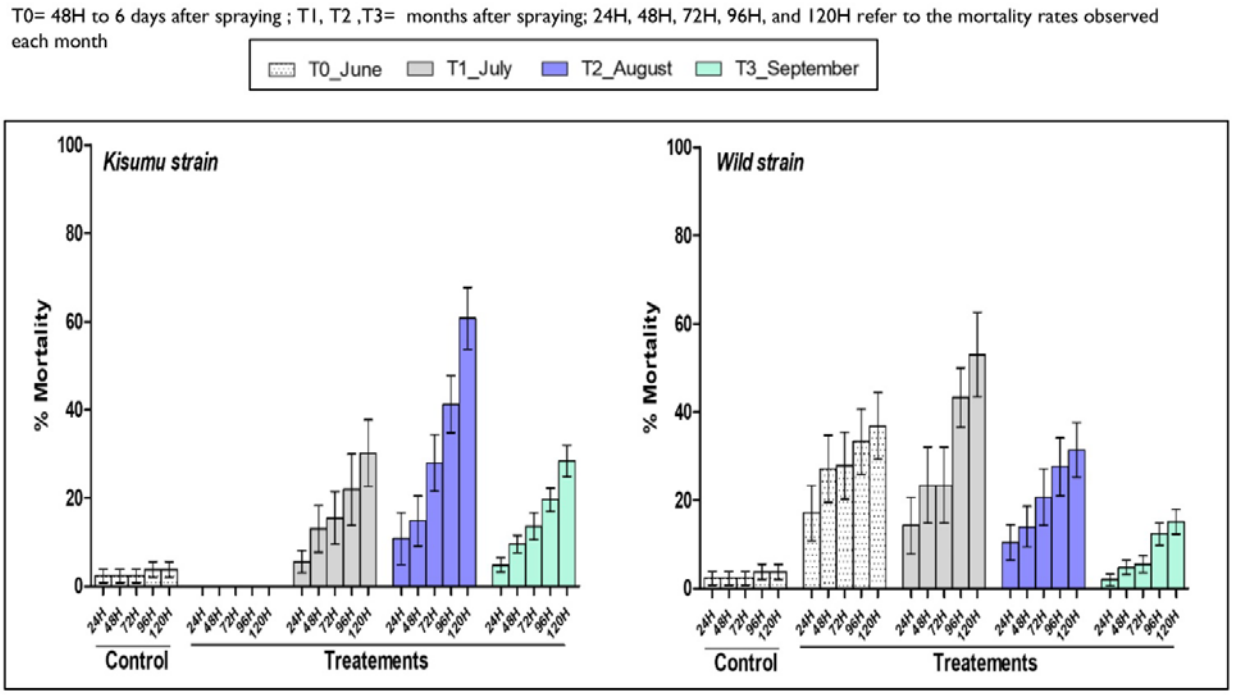
Fumigant assays performed in Kongoussi (sprayed with Actellic® 300CS) indicated varying, but generally low mortality rates obtained when mosquitoes were exposed for 30 minutes, one meter away from treated walls. The highest mortality rates were in June (T0) reaching a maximum of 55 percent in Mole, but this was followed by a progressive reduction during the next three months (July to September).

Figure 20: Fumigant assay on *Anopheles gambiae* "Kisumu" and *Anopheles gambiae* s.l. from Kongoussi sprayed with Actellic® 300CS.



Fumigant assays conducted in Kampti in houses sprayed with SumiShield WG indicated that there was some fumigant mortality, but it was slow acting and increased gradually over five days post exposure. The highest fumigant mortality was around 50 to 60 percent (five days after exposure) for Kisumu and wild mosquitoes. Three months after spraying, mortality rates were low at 20 to 30 percent (Figure 21).

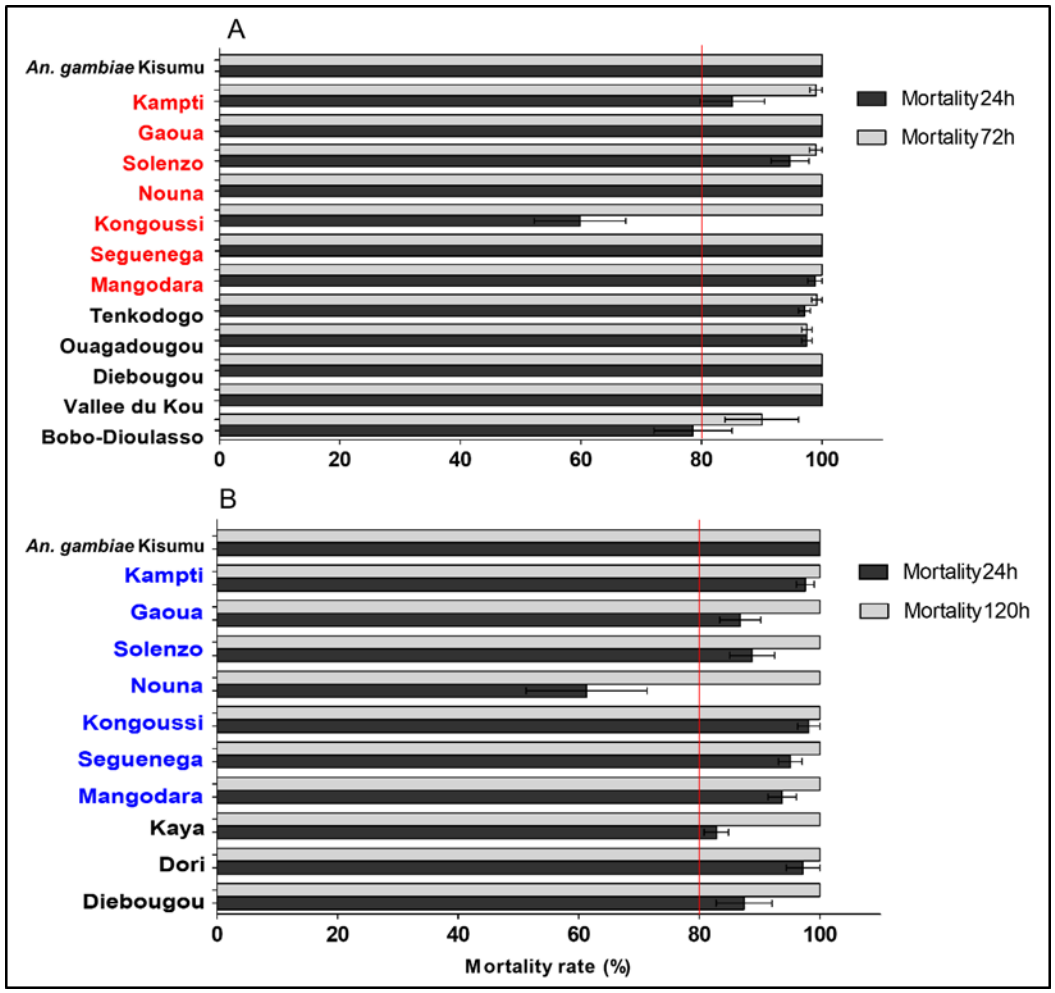
Figure 21: Fumigant assay on *Anopheles gambiae* "Kisumu" and *Anopheles gambiae* s.l. from Kampti sprayed with SumiShield® 50WG.



2.10 INSECTICIDE SUSCEPTIBILITY DATA

Figure 22 summarizes the results of susceptibility tests performed with local malaria vectors (*An. gambiae* s.l.) against chlorfenapyr 100ug/bottle and clothianidin 2 percent. There was high mortality (98-100%) only 24 hours after exposure with chlorfenapyr 100ug/bottle, except in Bobo Dioulasso, Kampti, and Kongoussi with less than 80 percent mortality rates. However, after 72 hours tests in all sites reached 98 to100 percent, except in Bobo-Dioulasso where it remained less than 95 percent (Figure 22A). Mortality rates obtained 24 hours after exposure with clothianidin 2 percent varied between 60 percent in Nouna (the lowest) and 98 percent (the highest) in Kampti, Solenzo, and Kongoussi. After 120 hours, all sites reached 100 percent mortality (Figure 22B).

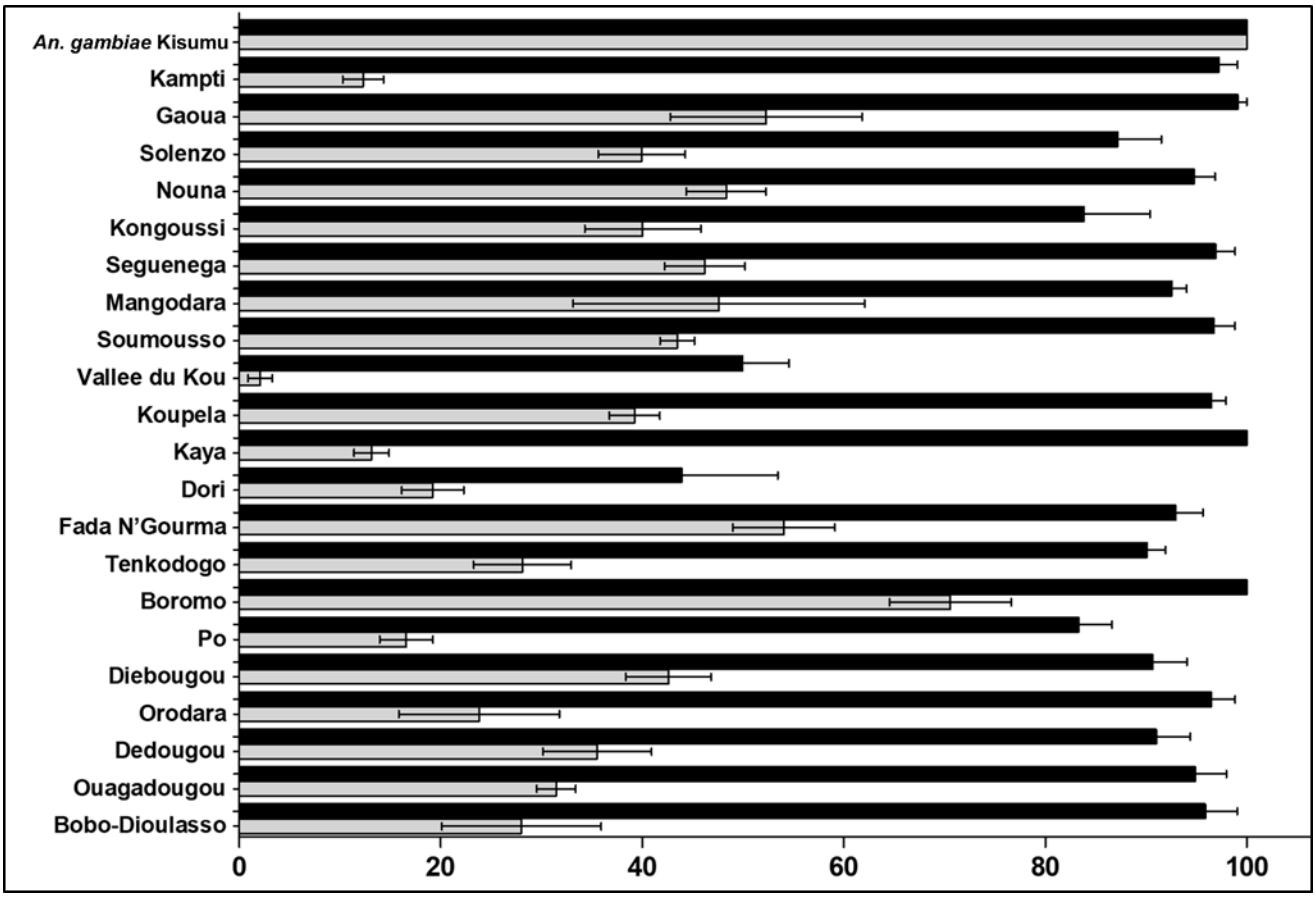
Figure 22: Susceptibility tests of wild *An. gambiae* s.l. to chlorfenapyr 100ug/bottle (250ml) and clothianidin 2% (h). Red and blue colors are referring to PMI IRS and control sites (plus Mangodara) respectively for chlorfenapyr and clothianidin testing. The blac for other sites completing the tests.



PMI sites were recorded in red for chlofenapyr (mortality rates were read at 24h and 72h) and blue for clothianidin (mortality rates were read at 24h and 120h).

The second set of data shows the results of nationwide susceptibility tests performed with deltamethrin (0.05%) as well as synergist assays with PBO 4% + deltamethrin (0.05%) (Figure 23). *An. gambiae* s.l. were resistant to deltamethrin in all sites nationwide. Pre-exposure to PBO followed by deltamethrin resulted in much greater mortality rates. The increase in mortality was substantial, with mortality rates reaching >80 percent in most sites.

Figure 23: Susceptibility tests of *Anopheles gambiae* s.l. against deltamethrin 0.05% (grey bar) and PBO 4% + deltamethrin 0.05% (black bar).



An. gambiae s.l. were also resistant to permethrin nationwide with mortality rates varying substantially. The lowest mortality rates were observed in Bobo-Dioulasso with less than 5 percent (Figure 24A). Nationwide monitoring with bendiocarb 0.1 percent indicated resistance in the South and Western areas in districts such as Mangodara, Soumousso, Po, Kampti, Gaoua, and Orodara, while most sites from the Center, East, and Northern parts were susceptible (Figure 24B).

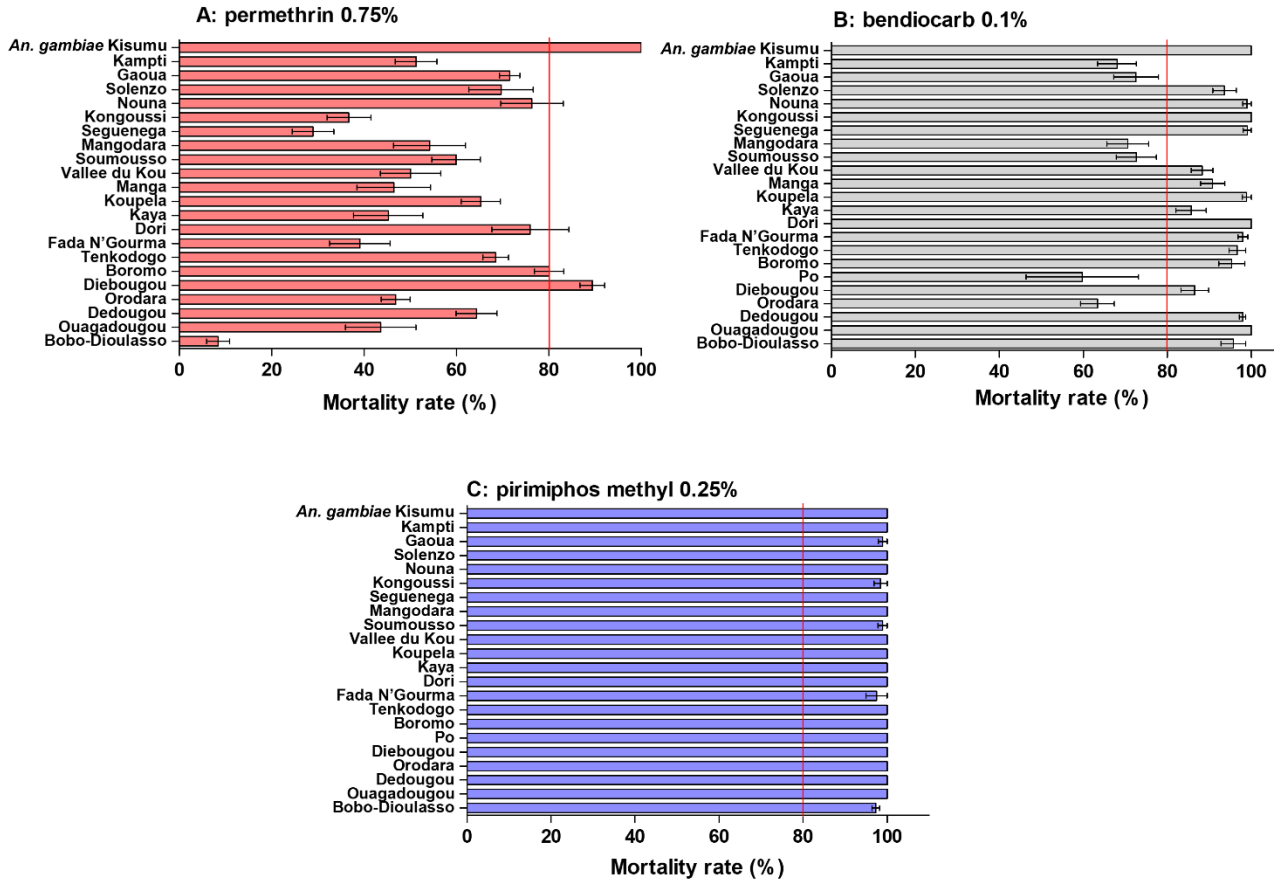
The susceptibility tests performed with pirimiphos-methyl against *An. gambiae* s.l. indicated that *An. gambiae* s.l. were susceptible in all sites, with mortality ranging between 98 to100 percent (Figure 24C). However, in 2017, several tests in the South and Western sites showed resistance in Gaoua, Kampti, and Mangodara.

These results suggested two hypotheses:

- The impregnated papers from 2017 with pirimiphos-methyl were underdosed.
- The impregnated papers of 2018 were overdosed and killed mosquitoes even though they were resistant.

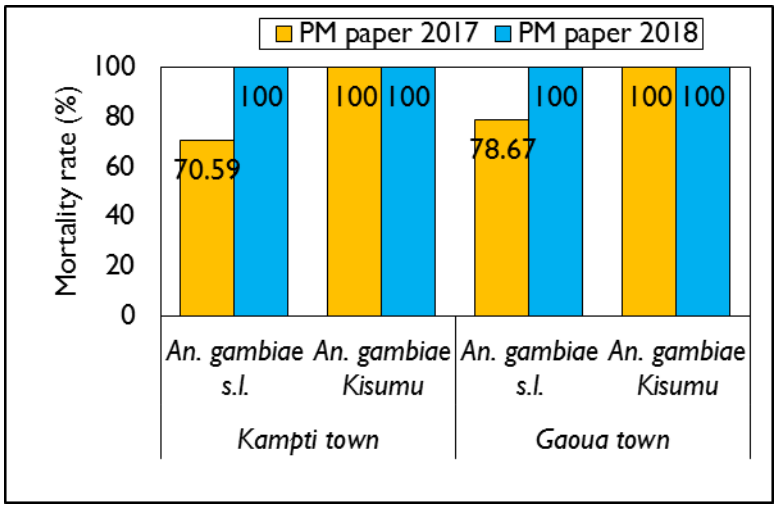
The pirimiphos-methyl papers have since been sent to a testing facility in Belgium for chemical analysis to determine whether the doses are as specified. Results are expected by July 2019.

Figure 24: Susceptibility tests of *Anopheles gambiae* s.l. against A) permethrin 0.75%, B) bendiocarb 0.1%, and C) pirimiphos-methyl 0.25% performed nationwide.



In 2018, the team conducted additional tests to confirm the results with the two batches of papers using *An. gambiae* s.l. from Kampti and Gaoua and *An. gambiae* Kisumu as the reference susceptible strain. The results presented in Figure 25 confirmed the high mortality rates obtained with 2018 papers and mortality rates less than 90 percent with the papers impregnated in 2017 (but still within their expiry date). To better address this question, we have sent samples of papers to Gembloux, Belgium for HPLC analysis of the insecticide content. Results are expected by June 2019.

Figure 25: Susceptibility of wild *An. gambiae* s.l. in Gaoua and Kampti tested with pirimiphos-methyl impregnated papers from 2017 and 2018.



2.11 Resistance Intensity (CDC Bottle Bioassay)

The results of resistance intensity tests for *An. gambiae* s.l. exposed in bottle bioassays to the diagnostic concentration of deltamethrin showed that mortality rates at the diagnostic dose varied between 78 to 100 percent (Table 7). The sites from the South West reached 100 percent mortality (Kampti, Gaoua, Mangodara, Bobo, and Nouna). Testing with twice the diagnostic concentration of deltamethrin did not result in 100 percent mortality for the remaining sites, particularly Ouagadougou, Solenzo, Kongoussi, and Seguenega with varying mortality rates ranging between 87.5 percent and 98 percent. The higher doses of 5x produced 100 percent mortality in Ouagadougou, Solenzo, and Kongoussi, but not in Seguenega with 95.6 percent. Overall, these findings indicate relatively low intensity of resistance to deltamethrin.

Table 7. Mortality of *An. gambiae* s.l. after 24h exposure to 1x, 2x, 5x concentrations of deltamethrin in CDC bottle bioassays and associated resistance intensity based on WHO classifications.

Sites	Deltamethrin diagnostic concentration (%)						Status
	1x (0.05)		2x (0.1)		5x (0.25)		
	n	% Mortality [IC]	n	% Mortality [IC]	n	% Mortality [IC]	
Kampti	48	100	48	100	NA		
Gaoua	44	100	47	100	NA		
Mangodara	61	100	59	100	NA		
Bobo-Dioulasso	44	100	52	100	NA		Low resistance intensity/no resistance
Nouna	47	100	57	100	NA		
Ouagadougou	59	94.02 [66.95-121]	62	98.48 [79.23-117]	61	100	
Solenzo	47	78.57 [33.19-124]	49	85.53 [7.68-163]	50	100	
Kougoussi	47	98.00 [72.59-123]	49	98.07 [73.64-122]	49	100	
Seguenega	47	83.97 [75.83-92]	49	87.54 [70.51-104]	44	95.65 [95.65-123]	***100% KD at 120mn***

A (Not applicable): 98–100% mortality at 2x dose indicates a low resistance intensity. Not necessary to assay at 5x dose.

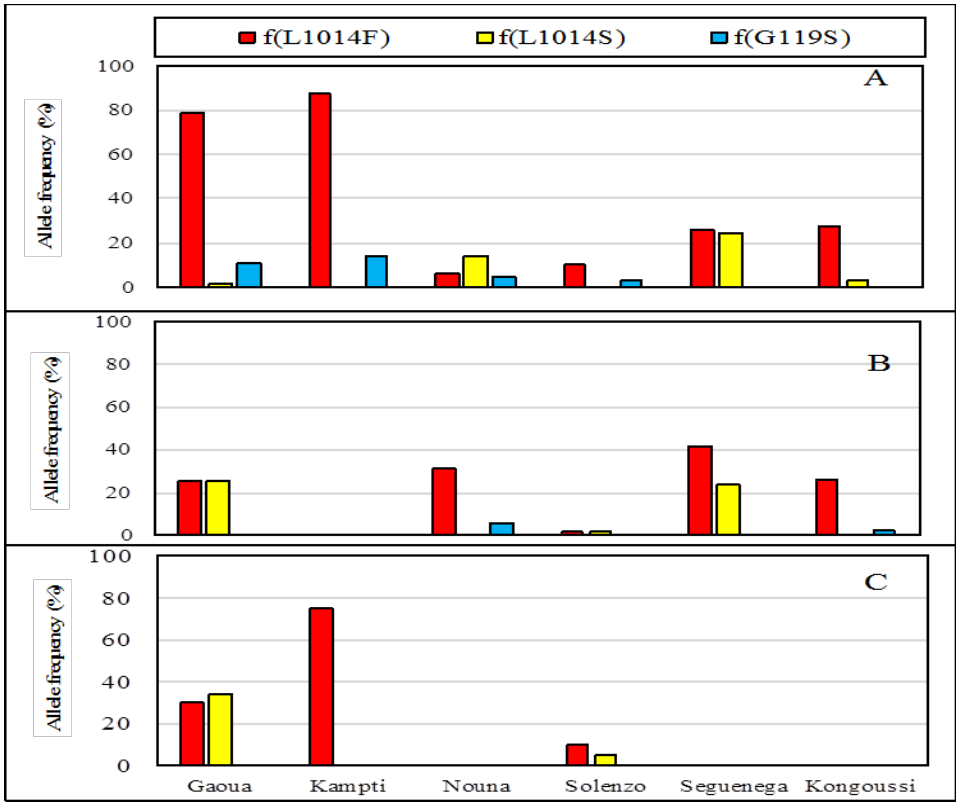
2.12 DISTRIBUTION OF ALLELE FREQUENCIES OF KDR (L1014F AND L1014S) AND ACE-1R MUTATIONS

The mean allelic frequency of the West African *kdr L1014F* mutation was 83 percent (Figure 27A) in *An. gambiae* populations from South Western sites (Gaoua and Kampti). It was low in Nouna and Solenzo (about 10%) and moderate in Kongoussi and Seguenega, averaging 28 percent. The *kdr* L1014S mutation was also found in *An. gambiae* populations (surprisingly) in Solenzo, Kongoussi, and Seguenega where the proportion of this species is very low. The gene frequency was also high in *An. coluzzii* populations in Gaoua, Solenzo, Kongoussi, and Seguenega in frequencies between 25 and 42 percent, whereas it was not found in the other sites (Figure 27B). The increasing frequency of the L1014S mutation indicates that selection for pyrethroid resistance is continuing. It is likely that a combination of multiple resistance mechanisms (L1014F, L1014S and metabolic resistance) may result in greater intensity of pyrethroid resistance which could impact efficacy of pyrethroid LLIN.

The *vgsc*-1014F allele was also found in *An. arabiensis* in Kampti in relatively high frequency of 75 percent and Gaoua at 30 percent and in very rare frequency in Solenzo (Figure 27C).

The *ace*-1R mutation was reported both in *An. gambiae* populations in four sites (Gaoua, Kampti, Solenzo, and Nouna) at relatively low frequencies, which did not reach 20 percent (Figure 27A). It was absent in the northern sites (Kongoussi and Seguenega). Ace-1R can cause resistance to organophosphates and carbamates; therefore, the low frequency of this gene is good news in the context of IRS with pirimiphos-methyl (an organophosphate).

Figure 26: Summary Allele frequencies of kdr (L1014 & 1041S) and ace-1R within *An. gambiae* (A), *An. coluzzii* (B) and (C) *An. arabiensis* populations from intervention and control sites.



3. CONCLUSIONS

- Cone bioassays with a susceptible insectary strain showed that both Actellic CS and SumiShield WG lasted for at least six months in all sites. However, tests with wild *An. gambiae* s.l. in Kongoussi produced reduced mortality rates with a mean residual efficacy of 4 months on concrete and 5 months on mud (>80% mortality), which may be an indication of pirimiphos-methyl resistance.
- Overall, *An. gambiae* s.l. biting rates were generally high across sites and biting rates were comparable between semi-urban sprayed sites as compared to their respective controls sites, with the exception of Kampti town where it was significantly lower.
- Peak biting rates for *An. gambiae* s.l. were observed in September practically in all sites, but more enhanced in the Center and Northern sites, which may be due to uncommon rainfall from August to September this year nationwide.
- Indoor and outdoor biting rates were generally similar in all sites.
- Parity rates showed minimal or no impact of IRS on mosquito age structure.
- Sporozoite rates were lower in Kampti, Solenzo, and Kongoussi towns as compared to their paired controls and may indicate that IRS with SumiShield WG and Actellic CS is having a positive impact on infection rates.
- The EIR was significantly lower in Kampti town compared to Gaoua town.
- EIR was also reduced in Kongoussi except in September where surprising transmission occurred decreasing the positive IRS impact. This situation was also observed in 2017 in the same site with a different insecticide.
- 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 in nearly all sites. PBO LLINs may provide greater control of malaria vectors in Burkina Faso than conventional pyrethroid LLINs.
- Full susceptibility of *An. gambiae* s.l. to pirimiphos-methyl was observed at all sites in 2018. This is in contrast to 2017 results, which showed resistance in Gaoua, Kampti, Mangodara, and Solenzo. WHO papers from 2017 and 2018 have been sent to the WHO collaborating center in Belgium for HPLC analysis to determine whether filter papers were under-dosed from 2017.
- Two new insecticides, chlorfenapyr and clothianidin, were also tested in PMI sites and *An. gambiae* s.l. was found to be fully susceptible at all sites. Interceptor G2 LLINs may also provide greater control than conventional pyrethroid LLINs.
- While IRS appears to be having some impact on malaria transmission, particularly by reducing sporozoite rates, the high biting rates and EIR in some IRS sites are concerning.
- While both SumiShield WG and Actellic CS produced good cone bioassay results, particularly with susceptible insectary mosquitoes, the impact of both insecticides on entomological indices was limited and there was no clear difference between the two formulations. The causes could be investigated to determine whether malaria vectors are avoiding sprayed surfaces (indoor resting rates, even in unsprayed sites, were generally much lower than biting rates) and also human behavior (the time people spend outdoors or unprotected by nets).

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5. ANNEX

Annex 1. Total catch size for all mosquito species collected by HLC (indoors and outdoors).

Study sites	***Anopheles* spp caught by HLC (indoors & outdoors)**					*Culex* spp	*Aedes* spp	*Mansonia*	**Total**
	An. gambiae s.l.	*An. nili*	*An. pharoensis*	*An. funestus*	*An. coustani*				
Gaoua town (Unsprayed)	3,456	1,220	22	24	6	1,151	41	21	5,941
Kampti town Sumishield WG	346	22	0	5	1	2,189	59	4	2,626
Kampti rural SumiShield	2,958	243	1	59	6	14	10	38	3,329
Nouna town (Unsprayed)	2,220	9	2	0	5	987	5	1	3,229
Solenzo town Shumishield WG	866	3	0	0	0	1,094	7	1	1,971
Solenzo rural Actellic CS	4,352	40	2	7	2	623	14	10	5,050
Seguenega town (Unsprayed)	1,079	6	0	0	0	6,593	27	1	7,706
Kongoussi town Actellic CS	928	2	0	0	6	347	23	13	1,319
Kongoussi rural Actellic CS	649	1	0	0	0	239	23	0	912
Total number (%)	17,034 *(52.8)*	1,546 *(4.79)*	27 *(0.08)*	95 *(0.29)*	26 *(0.08)*	13,237 *(41.03)*	209 *(0.65)*	89 *(0.28)*	32,263

Annex 2. Female *An. gambiae* s.l. collected by pyrethrum spray catches (PSC).

	Gaoua town unsprayed	Kampti town Sumishield WG	Nouna town unsprayed	Solenzo town Sumishield WG	Seguenega town unsprayed	Kongoussi town Actellic CS	Total
Unfed	12	7	12	12	24	9	76
Fed	346	96	275	354	688	123	1,882
Half gravid	17	4	8	14	20	2	65
Gravid	107	34	199	132	298	50	820
Total	**482**	**141**	**494**	**512**	**1,030**	**184**	**2,843**

Annex 3. Blood meal sources of *Anopheles gambiae* s.l., June to December 2018 per site

Month	Blood meal sources	Gaoua town unsprayed	Kampti town Sumishield WG	Nouna town unsprayed	Solenzo town Sumishield WG	Seguenega town unsprayed	Kongoussi town Actellic CS	Kampti rural Sumishield WG	Solenzo rural Sumishield WG	Kongoussi rural Actellic CS
June	Human	35	17	3	6	4	0	0	0	0
	Cattle	0	0	0	0	0	0	0	0	0
	Goat/sheep	1	0	0	3	0	0	0	0	0
	Pig	2	0	0	0	0	0	0	0	0
	Mixed human/animal	10	2	0	0	0	1	0	0	0
	Mixed animals	1	0	0	1	1	0	0	0	0
	Total	49	19	3	10	5	1	0	0	0
July	Human	34	9	16	4	23	18	14	12	15
	Cattle	0	0	0	0	1	0	0	0	0
	Goat/sheep	0	0	0	1	2	0	1	0	3
	Pig	0	0	1	0	0	1	0	0	0
	Mixed human/animal	12	6	1	4	6	1	15	7	5
	Mixed animals	1	0	15	3	13	3	4	10	2
	Total	47	15	33	12	45	23	34	29	25
August	Human	44	8	23	5	29	4	36	8	20
	Cattle	0	0	0	0	2	0	0	1	0
	Goat/sheep	0	0	0	1	6	2	0	4	0
	Pig	2	0	0	0	0	1	0	0	0
	Mixed human/animal	1	0	3	0	2	0	0	0	1
	Mixed animals	0	0	14	0	7	2	0	25	12
	Total	47	8	40	6	46	9	36	38	33

Month	Blood meal sources	Gaoua town unsprayed	Kampti town Sumishield WG	Nouna town unsprayed	Solenzo town Sumishield WG	Seguenega town unsprayed	Kongoussi town Actellic CS	Kampti rural Sumishield WG	Solenzo rural Sumishield WG	Kongoussi rural Actellic CS
September	Human	47	45	27	24	29	22	37	20	27
	Cattle	0	0	0	0	0	0	0	0	0
	Goat/sheep	0	0	2	1	6	4	0	1	1
	Pig	0	0	0	1	0	4	0	0	1
	Mixed human/animal	1	3	2	9	3	2	3	3	1
	Mixed animals	0	0	16	11	11	9	9	26	16
	Total	48	48	47	6	49	41	49	50	46
October	Human	39	4	26	5	28	6	41	25	12
	Cattle	0	0	0	0	2	0	0	0	0
	Goat/sheep	0	0	1	0	3	1	0	0	1
	Pig	0	0	0	0	1	1	0	0	0
	Mixed human/animal	4	0	0	0	6	1	5	0	1
	Mixed animals	0	0	4	10	7	4	2	8	2
	Total	43	4	31	6	47	13	48	33	16
November	Human	13	0	1	1	7	2	4	3	1
	Cattle	0	0	0	0	0	0	0	0	0
	Goat/sheep	0	0	0	0	0	1	0	1	0
	Pig	0	0	0	0	0	0	0	0	0
	Mixed human/animal	0	0	0	0	0	0	0	2	0
	Mixed animals	0	0	0	2	1	0	0	2	0
	Total	13	0	1	6	8	3	4	8	1
December	Human	4	0	0	0	0	2	0	0	5
	Cattle	0	0	0	0	0	0	0	0	0
	Goat/sheep	0	0	0	0	1	0	0	0	0
	Pig	0	0	0	0	0	0	0	0	0
	Mixed human/animal	0	0	0	0	0	0	0	0	1
	Mixed animals	2	0	0	0	1	0	0	0	1
	Total	6	0	0	6	2	2	0	0	7