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THE PMI VECTORLINK BURKINA FASO 2020 ENTOMOLOGICAL MONITORING ANNUAL REPORT

JANUARY – DECEMBER 2020

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

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CONTENTS

List of Figures

List of Tables

Annex

ACRONYMS

ANOVA	Analysis of Variance
CS	Capsule Suspension
EIR	Entomological Inoculation Rate
HBR	Human Biting Rate
HLC	Human Landing Catch
IRS	Indoor Residual Spraying
IRSS	Health Sciences Research Institute /Institut de Recherche en Sciences de la Santé
ITN	Insecticidal Treated Net
GLMM	Generalized Linear Mixed Models
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 that non-pyrethroid indoor residual spraying (IRS) should be used as a complementary vector control tool together with insecticide treated nets (ITNs) in locations where pyrethroid resistance occurs. In 2020, PMI VectorLink conducted spray operations from June 01-26, 2020 in Solenzo and Kampti districts using two insecticide formulations. The project sprayed clothianidin (SumiShield 50 WG) in Solenzo and a mixture of clothianidin and deltamethrin (Fludora® Fusion) in Kampti. Kongoussi district (which was sprayed in 2018) wasn't sprayed in 2020 partly due to security issues, but entomological monitoring was conducted. The project sprayed a total of 162,037 structures out of 171,276 eligible structures found by spray operators accounting for a final spray coverage rate of 94.6 percent. Previously, a nationwide mass distribution campaign supplied 1.5 million piperonyl butoxide (PBO)-synergist PermaNet 3.0 ITNs and two million dual-active ingredient Interceptor G2 ITNs, in addition to standard pyrethroid ITNs (PermaNet^{R} 2.0, DuraNet^{R}, MagNet^{R} and Interceptor^{R}) 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) was conducted in two sprayed sites (Solenzo and Kampti), with two paired unsprayed sites (Nouna and Gaoua), the former IRS site of Kongoussi plus adjacent control site (Seguenega) and also two sites (Karangasso-Vigué and Soumousso) where PBO ITNs were distributed. Insecticide susceptibility tests were conducted in 20 sites to determine the appropriate selection of insecticides for future IRS rounds and ITN distribution campaigns. Susceptibility tests using *An. gambiae* s.l. were conducted with organophosphates (pirimiphos methyl), pyrethroid insecticides and PBO synergists according to World Health Organization (WHO) protocols. CDC bottle bioassays were also conducted to determine susceptibility status to the newer insecticide chlorfenapyr. In addition, the team performed WHO tube tests to determine susceptibility to clothianidin, used for IRS.

Cone bioassays with a susceptible insectary strain showed that Fluroda Fusion WP-SB and SumiShield WG both lasted for at least seven months in all sites (tests are ongoing). Therefore, both formulations provided control throughout the peak malaria transmission season. *An. gambiae* was the predominant malaria vector species in the southwest (Gaoua, Kampti, Karangasso-Vigué), while *An. coluzzii* was more frequent in the north-central (Seguenega and Kongoussi) and west-central (Nouna and Solenzo) sites. The proportion of *An. arabiensis* 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 of indoor resting densities and biting rates was observed in September/October in all sites, approximately three to four months after spraying. Biting rates were greater in Solenzo (SumiShield WG) than in Nouna (unsprayed), and there was no clear difference in biting rates for Kampti (Fludora Fusion

WP-SB) rural site compared to Gaoua (unsprayed). When broken down to sub-locations, IRS appears to have more impact in central sub-locations than in rural sub-locations where densities are highest. The density of *An. gambiae* s.l. collected from PSC was generally slightly lower in IRS sites than in their paired unsprayed control sites, particularly in Kampti.

There was no clear reduction in the proportion of parous *An. gambiae* s.l. in sprayed sites compared to unsprayed sites. The mean malaria sporozoite rate (by PCR) was extremely high in southwestern locations, with a positivitiy rate in unsprayed Gaoua of 24.8% (133/536), and 20.0% (84/419) in neighboring Kampti (Fludora Fusion). In Nouna (unsprayed) in central-western Burkina Faso the sporozoite rate was lower at 10.5% (41/390) and was even lower in sprayed Solenzo (SumiShield) at 6.4% (25/391).

The entomological inoculation rate (EIR) was extremely high in the southwest, with Gaoua (unsprayed) having an EIR of 256 infective bites per person (indoors) for the 7 months between June and December 2020. The EIR was slightly lower in the neighboring sprayed district of Kampti (Fludora Fusion WP-SB) but was still high with 214 infective bites per person (indoors) for seven months. The EIR was also only slightly lower in the sprayed site of Solenzo with 186 infective bites per person, compared to 194 in unsprayed Nouna. Overall, IRS appears to have had some impact on EIR due to slightly lower sporozoite rates, but the malaria risk remains extremely high in both sprayed and unsprayed sites. In the two sites where PBO ITNs were distributed, the EIR was also high, with 86 infectious bites per person in Karangasso-Vigué and 87 in Soumousso (for seven months).

The susceptibility data showed full susceptibility of *An. gambiae* s.l. to pirimiphos-methyl and clothianidin at all IRS locations. Therefore, insecticide formulations containing pirimiphos-methyl and clothianidin can continue to be used in Burkina Faso for IRS in rotation. The insecticide susceptibility tests also revealed that *An. gambiae* s.l. were resistant to all pyrethroids tested, but pre-exposure to PBO increased mosquito susceptibility to alpha-cypermethrin, deltamethrin and permethrin in all sites tested, including Karangasso-Vigue and Soumousso, where Permanet 3.0 nets (PBOplus deltamethrin ITNs) were distributed in 2019. However, there were a few sites (Fada, Dori, Vallée du Kou, Kongoussi and Seguenega) where the increase in mortality due to PBO pre-exposure was consistently low for all three pyrethroids and PBO nets may not be suitable for these locations. Pyrethroid resistance intensity was high in all sites for deltamethrin and alpha-cypermethrin and moderate or high for permethrin. This highlights the notion that high resistance intensity throughout Burkina Faso may be undermining performance of pyrethroid-only ITNs. On a positive note, susceptibility to chlorfenapyr was recorded at all sites. Due to the widespread presence of pyrethroid resistance, PBO synergists and bi-treated nets such as Interceptor G2 (containing chlorfenapyr + pyrethroid), should continue to be prioritized for future distribution campaigns.

1. INTRODUCTION

The World Health Organization (WHO) reported 229 million malaria cases and 409,000 deaths worldwide in 2019 (WHO, 2020). Malaria is endemic in Burkina Faso, and the most recent National 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 National Malaria Control Program (NMCP) recorded approximately 11.9 million confirmed cases of malaria and 4,292 deaths reported by health facilities. This makes Burkina Faso the third most malaria affected country after the Democratic Republic of Congo and Nigeria (NMCP report, 2019). The primary malaria parasite in Burkina Faso is *Plasmodium falciparum* (Hien *et al*., 2017) which is primarily transmitted by *Anopheles gambiae* s.l. and *Anopheles funestus* (Dabiré *et al.,* 2007, 2012). The use of insecticide treated mosquito nets (ITNs) 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*-L1014F), 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 ITNs (Toé *et al*., 2015).

The national malaria strategic plan recommends that non-pyrethroid-based IRS be used as a complementary vector control tool together with ITNs 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 malaria pyrethroid resistant vectors. With US President's Malaria Initiative (PMI) funding, IRS was included as a priority vector control strategy and has been implemented annually since 2018. PMI VectorLink conducted spray operations from June 01-26, 2020, using clothianidin (SumiShield 50 WG) in Solenzo and a combination of clothianidin and deltamethrin (Fludora® Fusion) in Kampti. Kongoussi district was sprayed in 2018-19 but in 2020 was not sprayed due to security issues, but entomological monitoring was conducted. The project sprayed a total of 162,037 structures out of 171,276 eligible structures found by spray operators, accounting for a final spray coverage rate of 94.6 percent. In addition, a mass distribution campaign in 2019 supplied 1.5 million piperonyl butoxide (PBO)-synergist PermaNet 3.0 ITNs and two million dual-active ingredient Interceptor G2 ITNs, in addition to standard pyrethroid ITNs.

The VectorLink Burkina Faso team conducted surveys to monitor vector bionomics and insecticide susceptibility during the period of high malaria transmission (June to December 2020). 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 unsprayed control sites.

- Monitor the susceptibility of *An. gambiae* s.l. to permetrhin 0.75 percent, deltamethrin 0.05 percent and alphacypermethrin 0.05 percent (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 the WHO tube protocol.
- Determine the susceptibility level of the main malaria vectors, *Anopheles gambiae* s.l., to two relatively new insecticides clothianidin and chlofenapyr.
- 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.
- Provide data, recommendations, and 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 2020 in eight sites: two IRS sites (Solenzo and Kampti), two paired unsprayed control sites located approximately 50km away (Nouna and Gaoua), one former IRS site (Kongoussi) plus neighboring control site (Seguenega), and two sites where PBO ITNs were distributed in 2019 (Soumousso and Karangasso-Vigué). Monthly mosquito collections were conducted to measure entomological parameters of malaria transmission. These sites are located across the three ecological zones of Burkina Faso: Sudanian (West), Sudano-Sahelian (West Central) and Sahelian (North) ecological zones (Figure 1).

Anopheles mosquitoes were sampled using three 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 a more urban central site and a rural site. Cone bioassays were conducted on sprayed walls in the two IRS districts to measure the quality of IRS and residual efficacy.

Figure 1. Study sites for monthly longitudinal trapping by HLC and PSC (IRS sites, unsprayed control sites, former IRS site) and sites for resistance monitoring

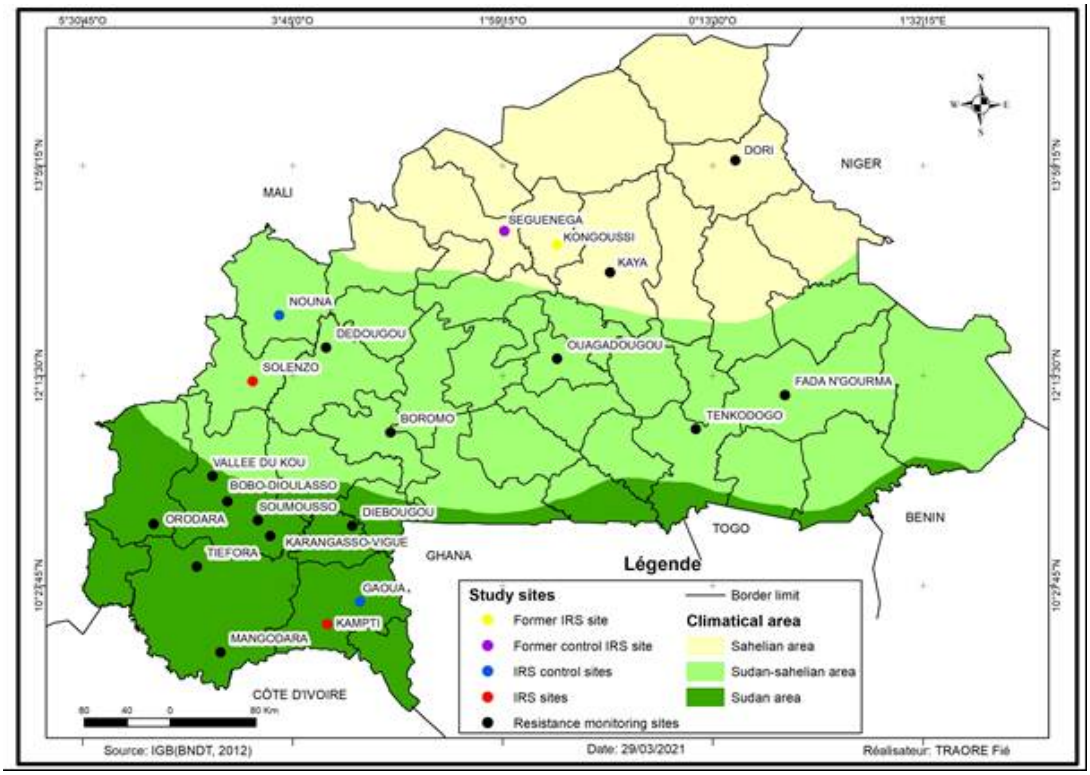
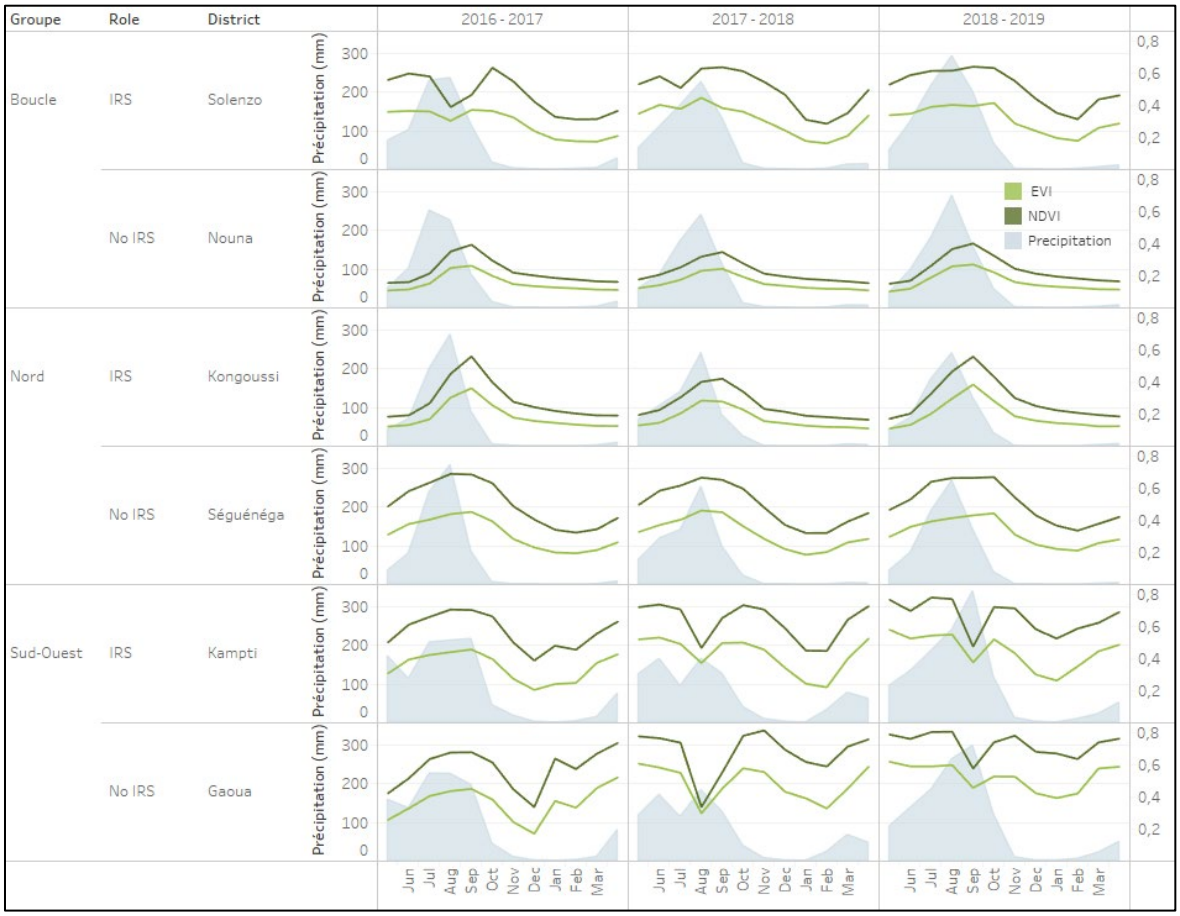


Figure 2. Average monthly precipitation (in millimeters) by district from 2016-2019



Source: Inouye et al (2020), Burkina Faso Indoor Residual Spraying 2018 Impact Evaluation.

The mean climate data for the two IRS sites Solenzo and Kampti including the former IRS site of Kongoussi (Figure 2) showed a relative short rainy season lasting approximately four months from June to September in Kongoussi and Solenzo and longer in Kampti, extending from April to October. The mean temperature was 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 per 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 *Anopheles* females 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 for further molecular laboratory analyses.

2.4 Parity Rates of *Anopheles* Females

Anophelines were morphologically identified to species level using taxonomic keys of Gillies and Coetzee (1987). A random sample of 50 unfed females per month collected from indoor and outdoor HLC were dissected. Ovaries were dissected to determine parity rate, by observing the coiling of ovarian tracheoles (Detinova and Gillies, 1964). All these specimens, including those dissected, were brought back to the Institute of Health Science Research/*Institut de Recherche en Sciences de la Santé* (IRSS) laboratory and stored at 4°C for further laboratory analyses.

2.5 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 2020 to December 2020 in IRS sites with WHO cone bioassays after spraying with SumiShield 50WG (Solenzo), 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.

2.6 LABORATORY ANALYSES

2.6.1 *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 infection 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).

2.6.2 Origin of Blood Meal Source (Anthropophily Rate)

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

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

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 (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.

2.6.4 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 and 200 µg/bottle (if necessary)

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

2.7 DATA ANALYSIS

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

The human biting rate (HBR) was determined as the number of mosquitoes collected by HLC divided by the number of collector-nights (indoors and outdoors). The *Anopheles* infection rate (IR) was calculated as the proportion of mosquitoes tested positive for *P. falciparum* DNA in the head or thorax. The entomological inoculation rate (EIR) was calculated as human biting rate multiplied by IR and estimated as the number of infectious bites per human during a period (day, month, annual). A Chi-square test with the R statistical software (Version 3.4.0) was used to compare the mortality rates between localities for susceptibility tests according to the insecticide tested. 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 models (GLMM) using the glmmTMB function. In the case of the count variables, we used negative binomial families like nbinom2. A difference was considered significant when the p-value was less than 0.05.

3. RESULTS

3.1 MALARIA VECTOR SPECIES COMPOSITION

From June to December 2020, a total of 25,960 anopheline mosquitoes were collected in all sites by HLC indoors and outdoors including 17,058 anopheline mosquitoes by HLC and 8,902 anopheline mosquitoes collected by PSC. *An. gambiae* s.l. was the most abundant species caught by HLC, with 16,278 (95.4 percent), followed by *An. nili* with 589 (3.5 percent) and a small proportion of *An. funestus* 128 (0.8 percent), *An. pharoensis* 54 (0.3 percent) and *An. coustani* 9 (0.05 percent) (Figure 3A). The abundance of *An. nili* and *An. funestus* was highest in Gaoua and Kampti in the Southwest from July to October and rare in Northern sites. *An. gambiae* sl was also the predominant species collected by PSC (8,798/8,902) (Figure 3B).

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

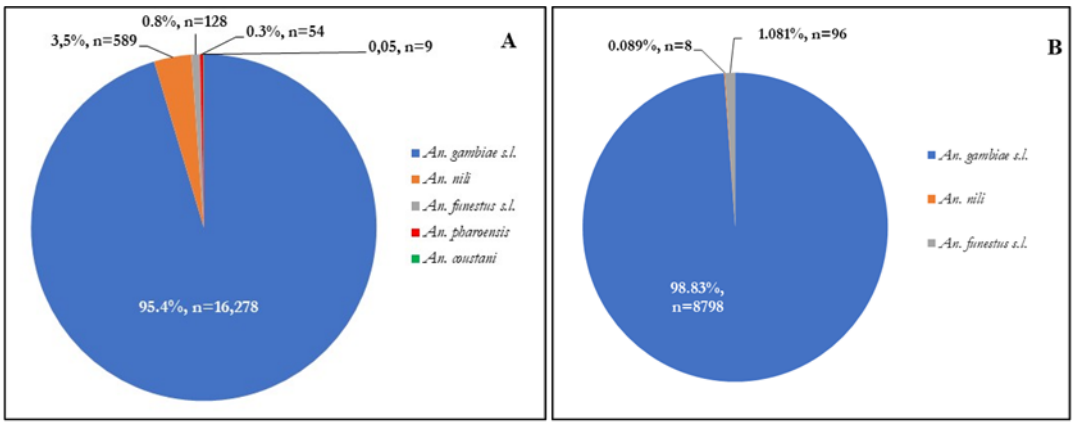
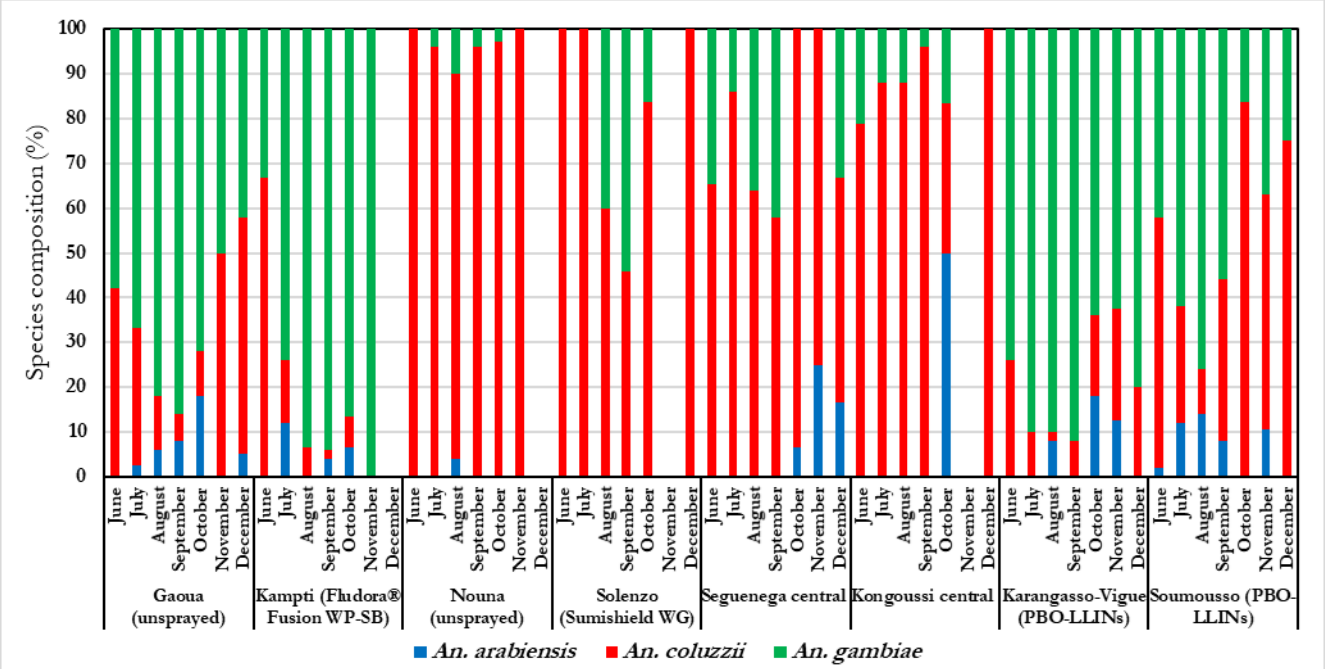


Figure 4 shows the species composition of the *An. gambiae* complex identified by PCR per site and by month from June to December 2020. *An. coluzzii* was the predominant species in Nouna, Solenzo, Seguenega and Kongoussi. The majority of *Anopheles* in Kampti and Gaoua were *An. gambiae* each month, except for June in Kampti and November and December in Gaoua where more *An. coluzzii* were collected. *An. arabiensis* were found every month at low proportions in Gaoua and Kampti, but the largest proportion at 50 percent was found in Kongoussi in October, although the proportion was low for other months. In Karangasso-Vigué (PBO net sites) *An. gambiae* was the predominant species for every month but in Soumousso the proportion of *An. gambiae* and *An. coluzzii* was generally equal, with more *An. coluzzii* collected later in the year in October-December, 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 (Kampti, Gaoua) 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), while

An. coluzzii was more frequent in the Centre North (Seguenega and Kongoussi) and Centre West (Nouna and Solenzo).

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

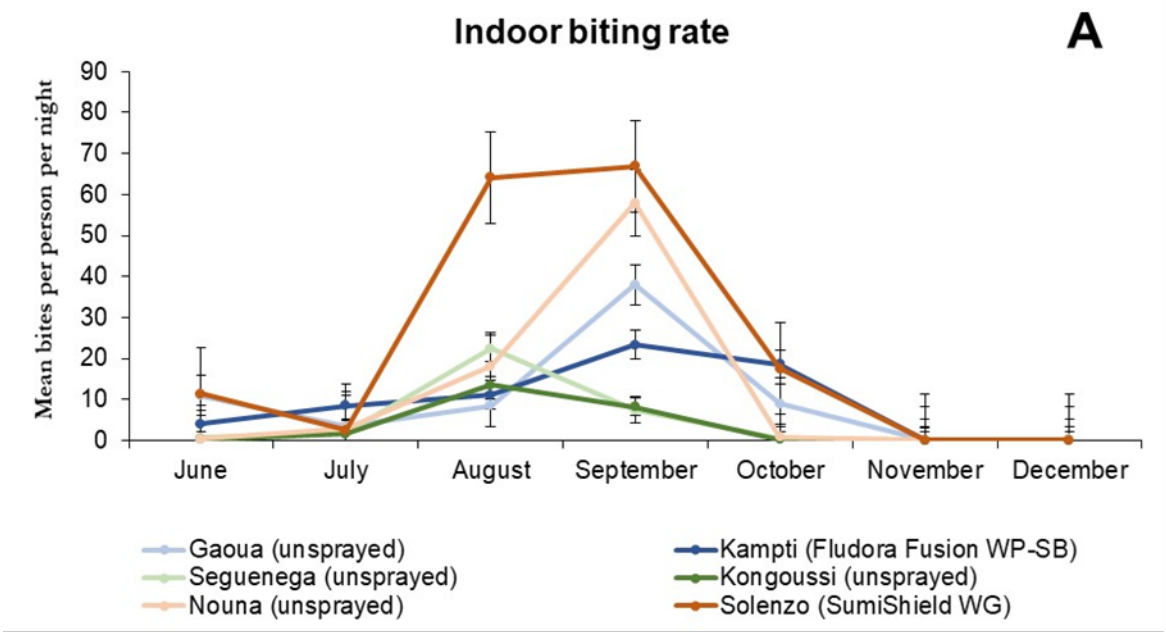


3.2 *AN. GAMBIAE* S.L. HUMAN BITING RATE (HBR)

Figure 5 presents the mean monthly indoor and outdoor *An. gambiae* s.l. human biting dynamics in all IRS sites and paired unsprayed control sites (combined data for 1 rural and 1 central sub-location). 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 Solenzo which had mean *An. gambiae* s.l. biting rates indoors and outdoors >5 bites/person/night (b/p/n). The biting rate increased monthly after July, reaching a peak 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 did not show any clear significant difference (Tukey's $p > 0.31$). While the impact of IRS on biting rates appeared to be minimal when the data were merged between rural and central sub-locations, there were consistently lower biting rates from July to December in the sprayed semi-urban central sites compared to paired unsprayed control sites (**Annex 1**). However, in rural sites, the biting rates were generally far higher than central sites irrespective of the region and there was yet no apparent impact of IRS on biting rates. The biting rates were particularly high in Solenzo (sprayed with SumiShield), which mainly was due to the rural sub-location which had numerous breeding sites and may not be comparable with the neighbouring control site of Nouna. Fortunately, in Kongoussi the biting rates remained relatively low, despite withdrawal of IRS, and were comparable with the neighboring control site of Seguenega. The biting trends were similar indoors and outdoors. From July to December, the biting

frequency in both PBO net sites increased monthly until a peak was reached in September at 26 bites per person per night indoors and 45 outdoors in Karangasso-Vigué (Figure 6).

Figure 5. Mean *An. gambiae* s.l. bites per person per night from indoor (A) and outdoor (B) HLC collections (combined central and rural sites) from June to December 2020



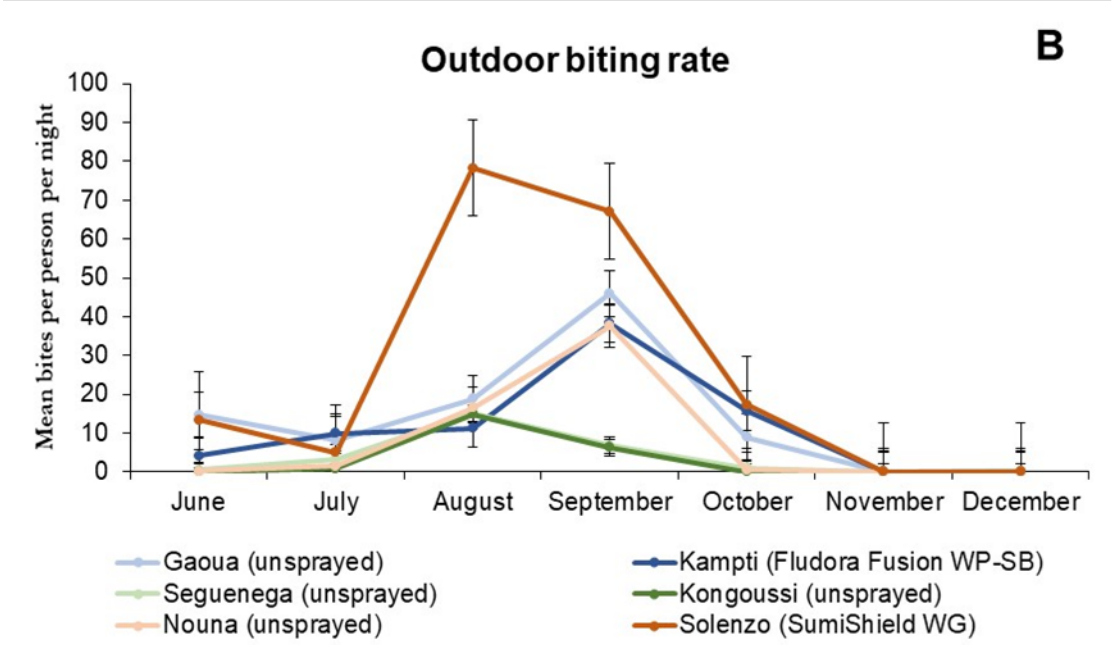
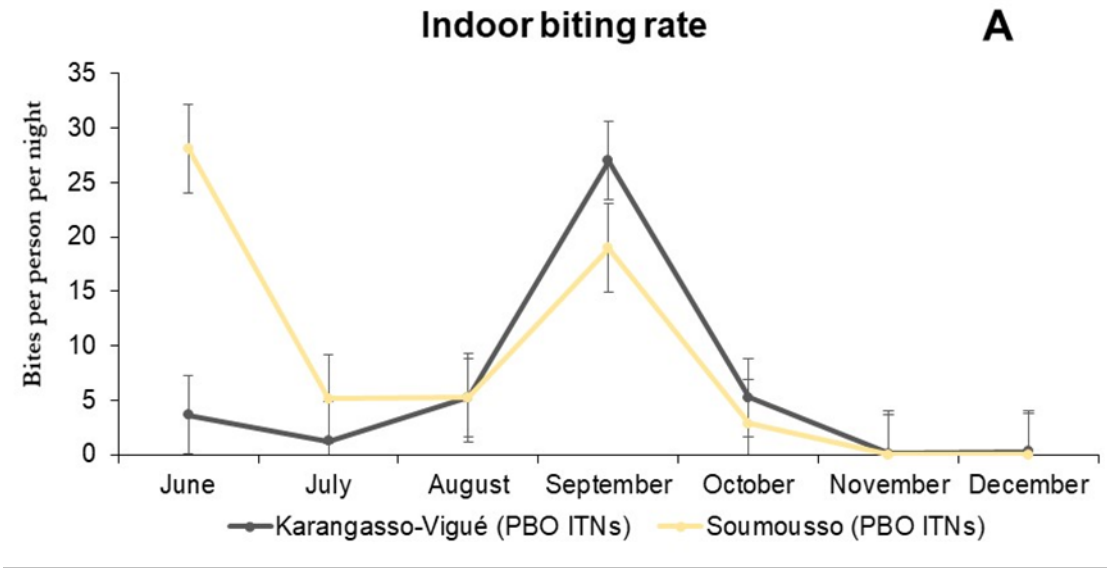
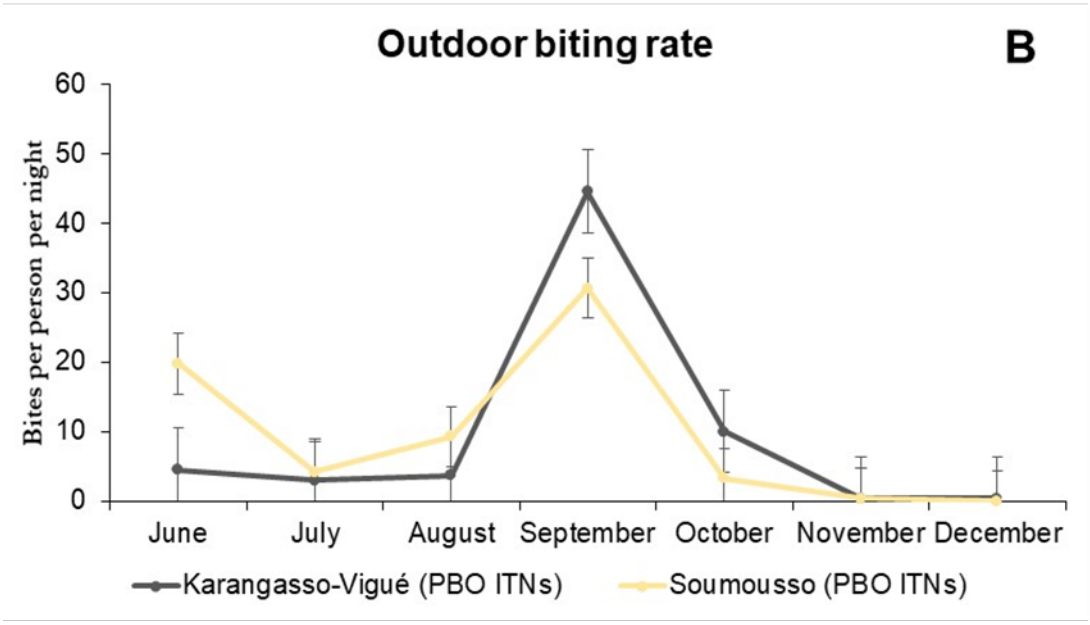


Figure 6. Mean *An. gambiae* s.l. bites per person per night indoors (A) and outdoors (B) from June to December 2020 in HLC collections from PBO ITNs sites





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

Figure 7 shows the mean hourly indoor and outdoor biting times of *An. gambiae* s.l. in IRS and unsprayed sites. The trends indoors (figure 7A) were broadly similar in all sites, except in Solenzo, with classic late night biting resulting in peak indoor and outdoor biting between 11pm and 5am. In Solenzo the mean number of bites was exceptionally high, peaking earlier at night between 10pm and midnight. In the two PBO ITN sites the biting intensity was similar between sites peaking between 10pm and 2am followed by a decrease

towards morning (Figure 8A) with a small proportion biting up to 7am in the morning outdoors (Figure 8B).

Figure 7. Mean number of Bites of *An. gambiae* s.l. per hour from June to December 2020 (cumulated data form central and rural sites): A) indoor and B) outdoor HLC collections

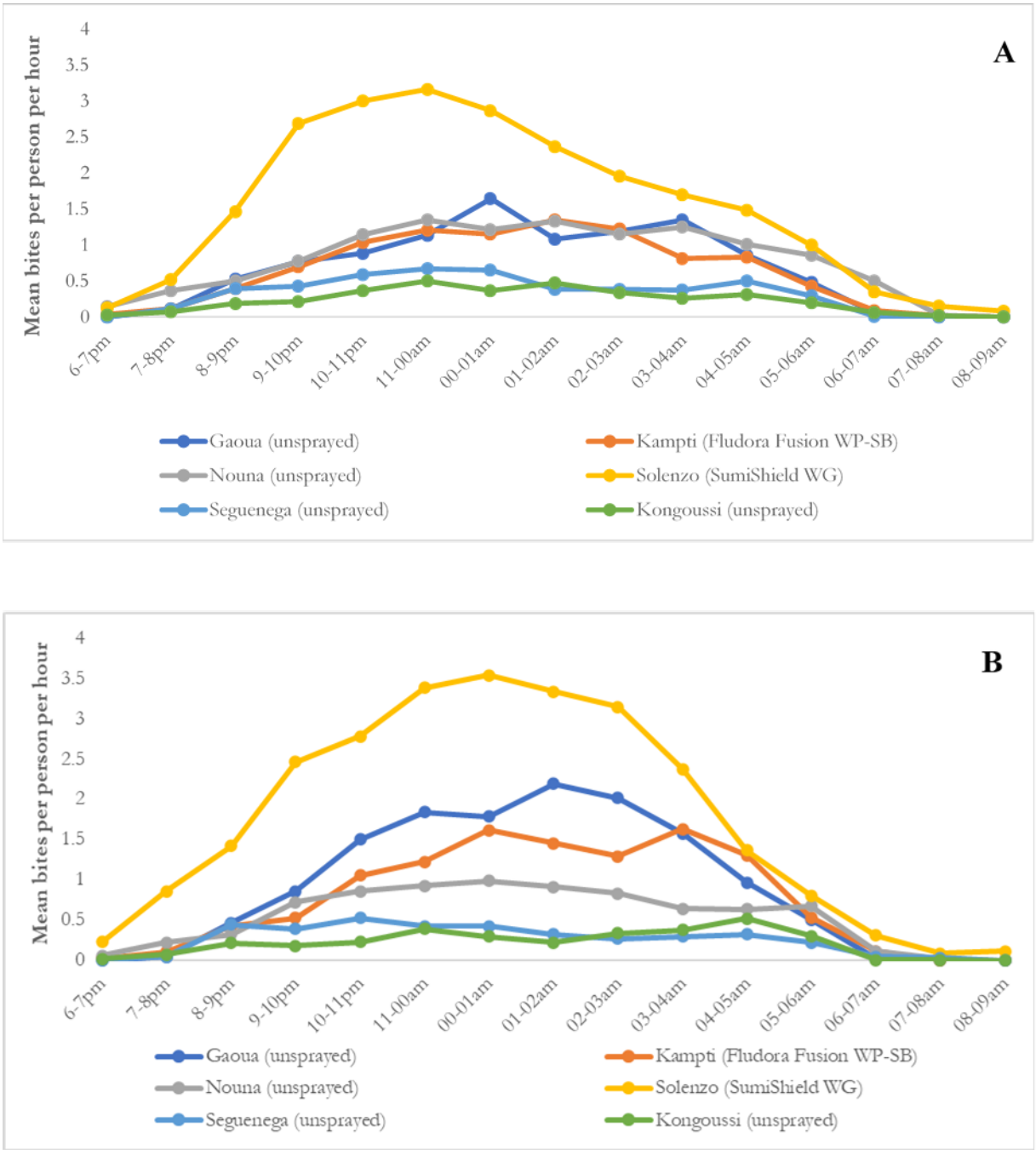
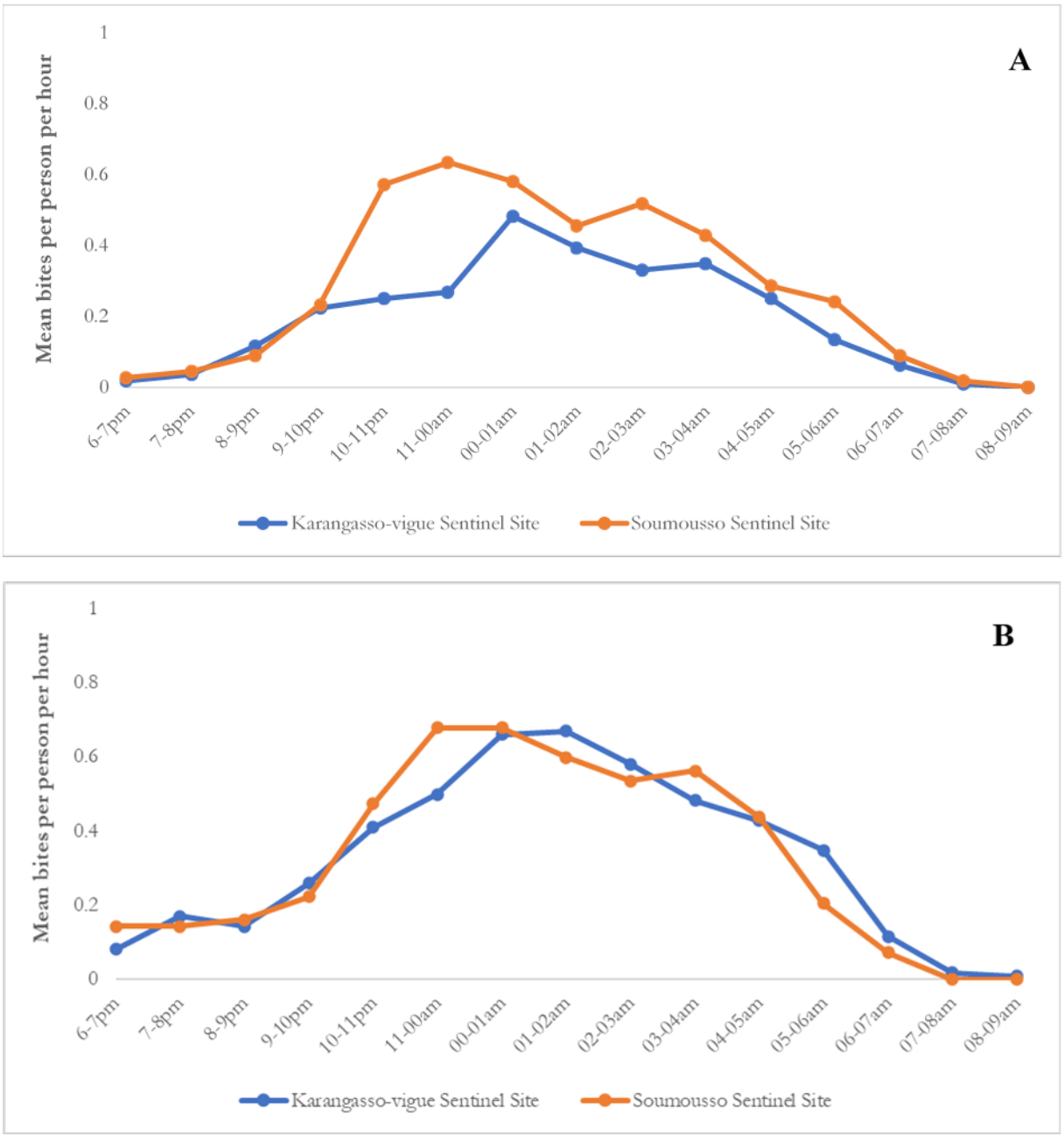


Figure 8. Mean number of *An. gambiae* s.l. bites per hour from A) indoors and B) outdoor HLCs from June to December 2020 in Soumousso and Karangasso-Vigue



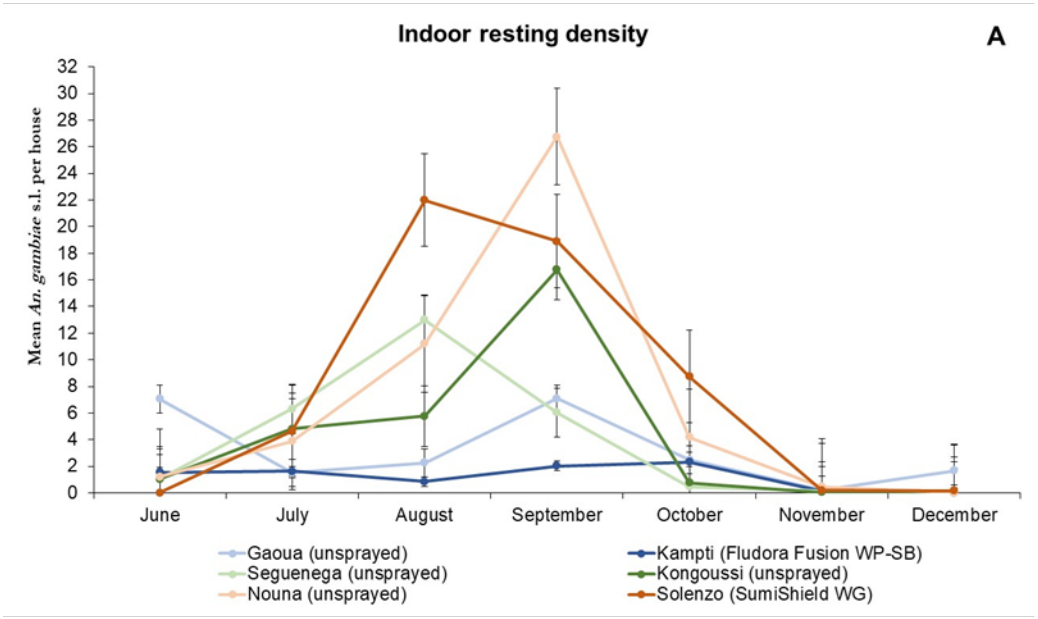
3.4 Indoor Resting Densities by PSC

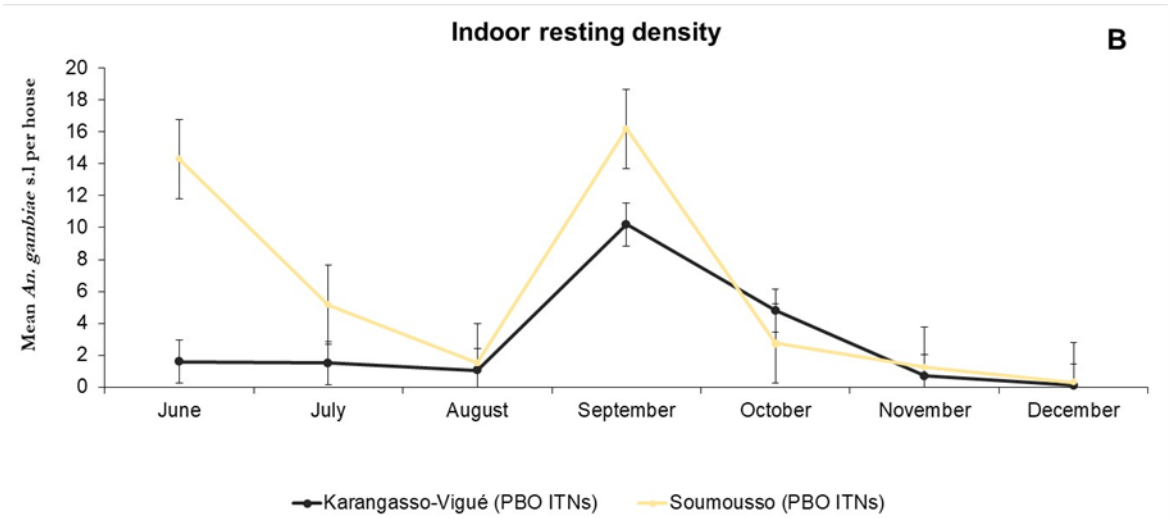
The density of *An. gambiae* s.l. collected from PSC was slightly lower in IRS sites than their paired unsprayed control sites (Figure 9A), particularly in Kampti where densities were always <6 *An. gambiae* s.l. per house per day. However, except Kampti, no statistically significant difference was observed between unsprayed control and IRS sites (Tukey's p> 0.42). In fact, in the sprayed site of Solenzo (SumiShield WG), the densities appeared to be no lower than in the paired unsprayed site of Nouna and reached a mean of 19 *An. gambiae* s.l. per day in September compared to 26 in Nouna, four months after IRS application. In Kongoussi, where

IRS was withdrawn, the resting density was similar to the neighbouring control site of Seguenega. The resting densities recorded in Soumousso were higher than those observed in Karangasso-Vigué with a peak in September in June and September (Figure 9B)

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 difference between IRS and control indoor resting densities.

Figure 9. Mean *An. gambiae* s.l. collected per house per month from indoor PSC in (A) IRS and unsprayed sites (combined central and rural) and (B) PBO ITN sites





3.5 PARITY RATE OF *ANOPHELES GAMBIAE* S.L.

Table 1 summarizes the overall parity rates from monthly dissected females between June and December 2020 by site. The parity rates did not differ significantly between sprayed and control sites in 2020, being sometimes higher in sprayed sites. In Kampti (sprayed) the parity rate was 65%, which was slightly lower than that of Gaoua (control site) with 67.7% (X^2=0.85, p = 0.35). In Kougoussi (former IRS site, last sprayed with SumiShield WG in 2018) the parity rate of 37.4% was significantly lower than its former control site of Seguenega at 74.3 % (X^2 = 62.6, p<0.001). However, in the sprayed district of Solenzo, it was significantly higher (70.4 %) than that of the unsprayed district of Nouna (58.3 %) (X^2 = 13.04, p = 0.003). There was no apparent difference in parity rates between PBO-ITNs sites Soumousso and Karanguasso-Vigué (X^2=0.86, p = 0.17).

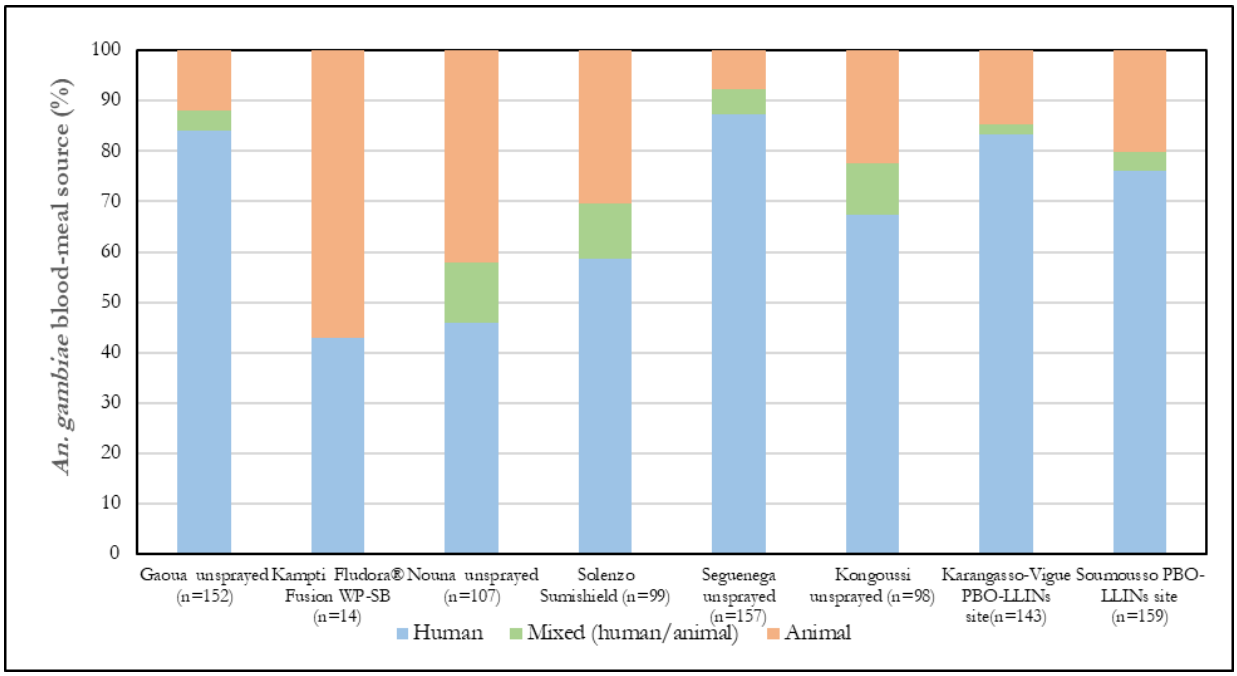
Table 1. Parity rate of *An. gambiae* s.l. females collected from sprayed sites (central and rural) and unsprayed sites (central and rural) between June and December 2020

Gaoua (unsprayed)	586	397	189	67.7
Nouna (unsprayed)	350	204	146	58.3
Seguenega (unsprayed)	261	194	67	74.3
Karangasso-Vigué (PBO ITNs)	114	89	25	78.0

3.6 *ANOPHELES GAMBIAE* S.L. BLOOD-MEAL SOURCE

An. gambiae s.l. were extremely anthropophilic in most of the study sites without any difference between sprayed and control sites, except in Kampti where a ratio 1:1 human and animal blood meals was recorded, although the number tested was very low (Figure 10). The proportion of strictly zoophagic *An. gambiae* reached 40% in Nouna percent and was lowest in Gaoua and Seguenega at around 10%. Mixed human and animal blood meals were not very common and was no greater than 10% of samples tested. The proportion of blood-meals that contained human blood (including mixed blood-meals) ranged from around 50% in Nouna to 90% percent in Seguenega and Gaoua.

Figure 10. Blood-meal source of *An. gambiae* s.l. collected by PSC between June and December 2020



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

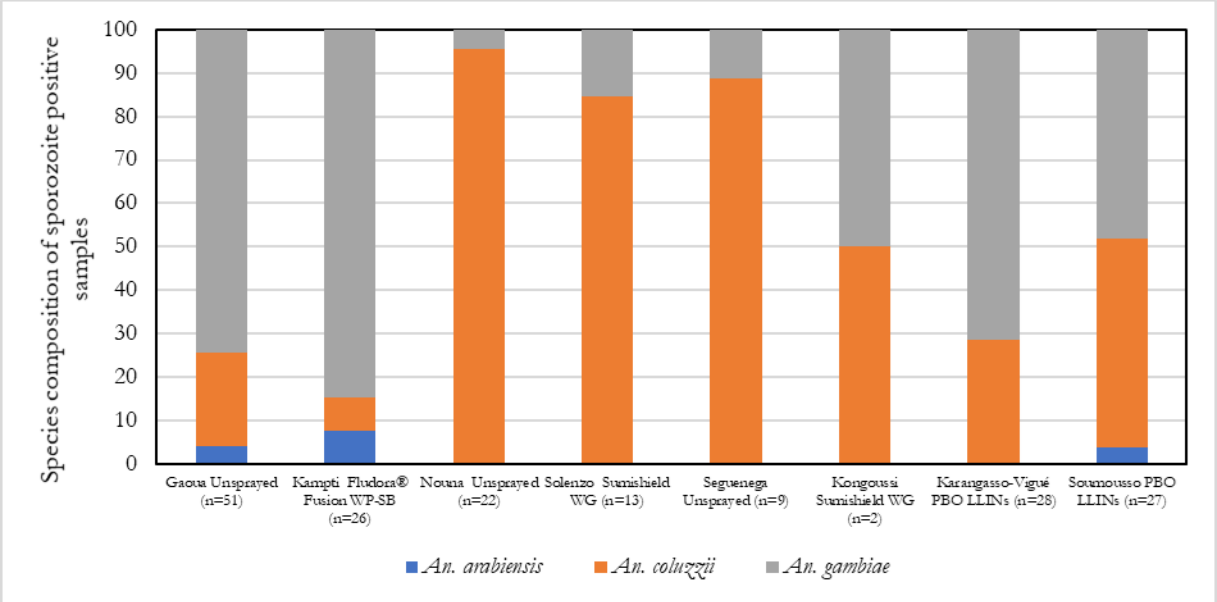
Irrespective of the vector control intervention the *P. falciparum* infection rates (IF) of *An. gambiae* s.l. in Burkina Faso remain higher in the West and South West parts and lowest in the Northern part (Table 2). With regard to IRS impact, the mean infection rates were significantly lower in sprayed sites than unsprayed sites. In the rural sublocations the infection rates were higher than in the central sites with some values reaching 30 percent in Gaoua and Kampti (Annex 4). The overall infection rates did not differ significantly between Soumousso and Karangasso the two PBO ITNs sites.

Table 2. *P. falciparum* infection rate (*IF value in red in the table*) recorded from indoor and outdoor HLCs collected *An. gambiae* s.l. per site from June to December 2020 (n= positive/Number tested)

Location	Site	IF indoors	IF outdoors	Total positve
South-west	Gaoua (unsprayed)	**22.93** (64/279)	**13.84** (69/257)	**24.81** (133/536)
South-west	Kampti (Fludora Fusion WP-SB)	**20.40** (40/196)	**19.73** (44/223)	**20.04** (84/419)
North-west	Nouna (unsprayed)	**15.02** (36/239)	**3.31** (5/151)	**10.48** (41/390)
North-west	Solenzo (SumiShield WG)	**7.17**(15/209)	**5.49**(10/182)	**6.39** (25/391)
North	Seguenega (unsprayed)	**6.22** (15/241)	**6.21** (9/145)	**6.21** (24/386)
North	Kongoussi (unsprayed)	**2.93** (8/273)	**6.09** (5/82)	**3.66** (13/355)
West	Karangasso-Vigué (PBO ITNs)	**13.16** (20/152)	**6.78** (8/118)	**10.37** (28/270)
West	Soumousso (PBO ITNs)	**9.32** (15/161)	**10.71** (12/112)	**9.89** (27/273)
	Overall Mean	**12.17**(213/1750)	**12.75**(162/1270)	**12.41** (375/3020)

Anopheles species composition was determined for those samples that were positive for *P. falciparum* sporozoites (Figure 11). Of those mosquitoes that tested positive for presence of *P. falciparum* DNA, between 50-85% were *An. gambiae* in the South-west sites of Gaoua and Kampti and western sites of Karangasso-Vigué and Soumousso. *An. coluzzii* was the main vector species transmitting malaria in Solenzo, Nouna, Kongoussi and Seguenega. Infected *An. arabiensis* were detected in Gaoua, Kampti and Soumousso but in low proportions between 2-8%.

Figure 11. Species composition of *P. falciparum* infected female *An. gambiae* s.l. from June to December 2020



3.8 ENTOMOLOGICAL INOCULATION RATE

The entomological inoculation rate was calculated for the period from June to December 2020 covering seven months (Table 3). The EIR was higher in the Southwest region particularly in Gaoua (unsprayed) with 256 infectious bites per person during this period indoors. The EIR was slightly lower in the IRS site of Kampti treated with Fludora Fusion compared to Gaoua its control unsprayed site with 214 infected bites (X^2 = 0.99, p=0.17). However, the outdoor EIR was higher in Gaoua than Kampti (X^2 = 51, p < 0.0001). The indoor EIR was similar in Solenzo (sprayed) and Nouna (unsprayed with 186 and 194 infectious bites respectively over 7 months (X^2=6.39, p = 0.06). In contrast the outdoor EIR was higher in Solenzo compared to Nouna (X^2=9.08, p =0.002).

In Kongoussi (IRS withdrawn), the EIR estimated indoors was the lowest with 11 infectious bites per person, compared to Seguenega with 33 infectious bites (X^2=1.98, p = 0.15). In the two PBO nets sites the EIR was similar indoors (X^2=9.91, p = 0.13) but the outdoor EIR were higher in Soumousso with 115 infected bites per person compared to Karangasso-Vigue with 72 infected bites (X^2=4.33, p = 0.03).

The EIR in rural sites either in sprayed or unsprayed sites were consistently high and despite some impact of IRS the risk of malaria in rural sites remained very high (Table 4).

Table 3. Entomological Inoculation Rate (June to December 2020) from combined central and rural data

	Gaoua (unsprayed)	Kampti (Fludora Fusion WP-SB)	Nouna (unsprayed)	Solenzo (SumiShield WG)	Seguenega (unsprayed)	Kongoussi (unsprayed)	Karangasso-Vigué (PBO ITNs)	Soumousso (PBO ITNs)
Total *An. gambiae* s.l. collected in indoor (HLC)	1,128	1,051	1,301	2,565	537	380	327	472
HLC trap-nights	112	112	112	112	112	112	112	112
HBR per night	10	9.375	11.61	22.90	4.79	3.39	2.92	4.21
Total *An. gambiae* s.l. tested by PCR (HLC)	279	196	239	209	241	273	152	161
Sporozoite rate	22.93%	20.40%	15.06%	7.17%	6.22%	2.93%	13.15%	9.31%
EIR per night	2.29	1.91	1.73	1.66	0.29	0.099	0.8	0.80
EIR indoor June to December 2020 (112 nights) (calculated using mean sporozoite rate)	**256**	**214**	**194**	**186**	**33**	**11**	**90**	**90**
Total *An. gambiae* s.l. collected in outdoor (HLC)	1,543	1,268	889	2,939	424	356	546	550
HLC trap-nights	112	112	112	112	112	112	112	112
HBR per night	13.77	11.32	7.93	26.24	3.78	3.18	4.87	4.91
Total *An. gambiae* s.l. tested by PCR (HLC)	257	223	151	182	145	82	118	112
Sporozoite rate	26.84%	19.73%	3.31%	5.49%	6.21%	6.09%	6.78%	10.71%
EIR per night	3.71	2.23	0.27	1.42	0.24	0.19	0.64	1.03
EIR outdoor June to December 2020 (112 nights) (calculated using mean sporozoite rate)	**415**	**250**	**30**	**159**	**27**	**21**	**72**	**115**

Table 4. Entomological Inoculation Rate (June to December 2020) from central sites (sprayed and unsprayed sites)

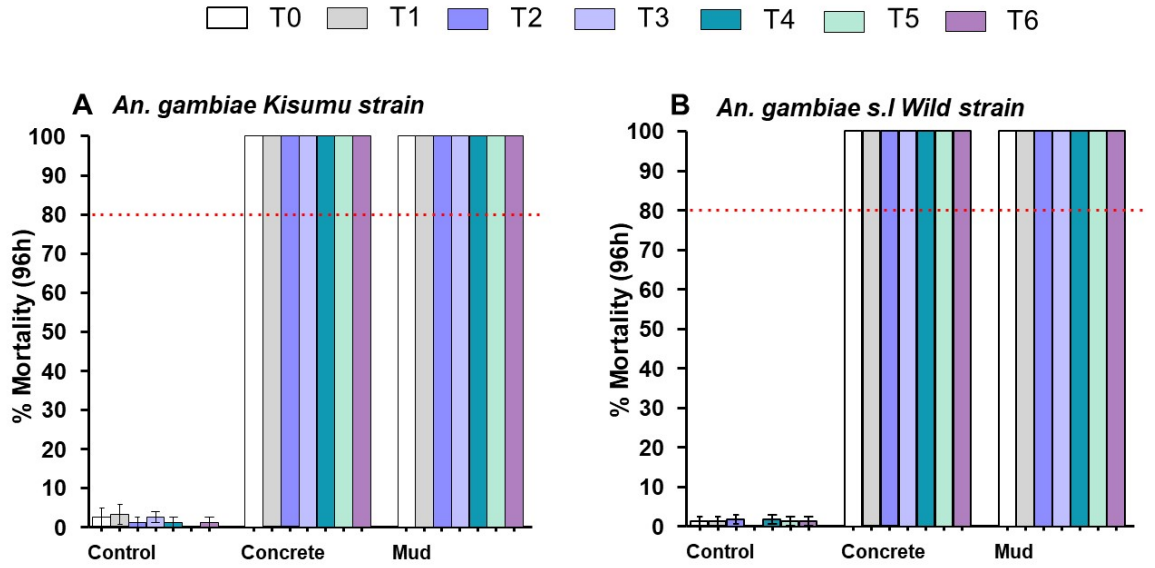
	Gaoua (unsprayed)	Kampti (Fludora Fusion WP-SB)	Nouna (unsprayed)	Solenzo (SumiShield WG)	Seguenega (unsprayed)	Kongoussi (unsprayed)	Karangasso -Vigué (PBO ITNs)	Soumousso (PBO ITNs)
Total *An. gambiae* s.l. collected in indoor (HLC)	431	165	766	666	184	230	342	483
HLC trap-nights	112	112	112	112	112	112	112	112
HBR per night	7.51	2.95	13.68	11.89	3.28	4.10	6.11	8.62
Total *An. gambiae* s.l. tested by PCR (HLC)	178	73	127	93	134	145	152	161
Sporozoite rate	20.02%	19.18%	15.74%	7.52%	5.97%	1.38%	13.15%	19.31%
EIR per night	1.53	0.57	2.15	0.89	0.19	0.056	0.80	0.80
EIR indoor June to December 2020 (112 nights) (calculated using mean sporozoite rate)	**171**	**64**	**241**	**99**	**21**	**6**	**90**	**90**
Total *An. gambiae* s.l. collected in outdoor (HLC)	581	335	486	903	254	289	531	539
HLC trap-nights	112	112	112	112	112	112	112	112
HBR per night	10.37	5.98	8.68	16.12	4.53	5.16	9.48	9.62
Total *An. gambiae* s.l. tested by PCR (HLC)	105	105	69	70	67	46	118	112
Sporozoite rate	14.28%	11.43%	5.79%	8.57%	2.98%	4.36%	6.78%	10.71%
EIR per night	1.48	0.68	0.50	1.38	0.135	0.22	0.64	1.03
EIR outdoor June to December 2020 (112 nights) (calculated using mean sporozoite rate)	**166**	**76**	**56**	**155**	**15**	**25**	**72**	**115**

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 (T0) to December 2020 (T6), the residual efficacy of each treatment (SumiShield 50WG and Fludora Fusion WP-SB), in terms of mosquito mortality, was evaluated by WHO cone tests using the susceptible "Kisumu" and wild *An. gambiae* s.l. from Solenzo and Kampti. For all tests the mortality rates of control bioassays on unsprayed walls were less than 5 percent.

In Solenzo district, the mortality rates (96h) with SumiShield 50WG reached 100 percent over a period of six months with walls made with mud and cement both for Kisumu and wild *An. gambiae* s.l. (Figure 12).

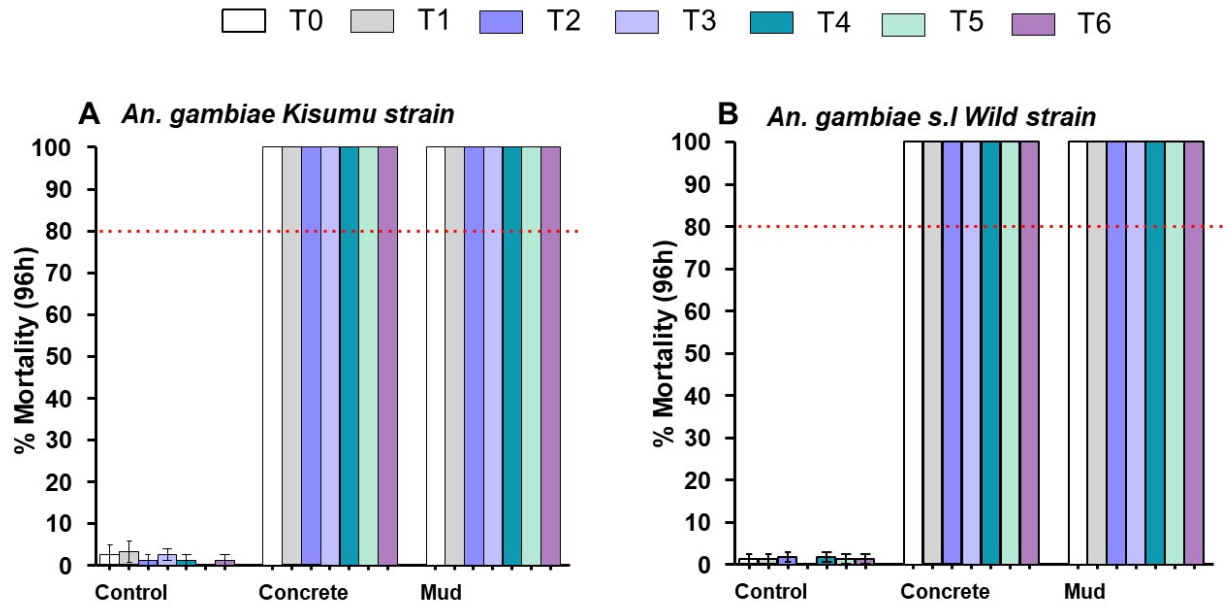
Figure 12. Mean mortality rates (96h) of *Anopheles gambiae* "Kisumu" (A) and wild *Anopheles gambiae* s.l (B) using monthly WHO cone bioassay on sprayed walls with SumiShield® 50WG in Solenzo district



Note: Error bars represent the 95% confidence interval. Red dashed line represents the WHO threshold of 80% mortality. T0 to T6: = 0 to 6 months after spraying.

In Kampti district, 100% mortality of both *An. gambiae* "Kisumu strain" and wild *An. gambiae* s.l was observed on cement and mud walls, (Figure 13). However, mortality rates of wild *An. gambiae* s.l on mud walls decreased to 98.61% in December (T6) (Figure 13).

Figure 13. Mortality rate (120h) of *Anopheles gambiae* "Kisumu" (A) and wild *Anopheles gambiae* s.l (B) using monthly WHO cone bioassay of sprayed walls with Fludora® Fusion in Kampti district



Note: Error bars represent the 95% confidence interval. Red dashed line represents the WHO threshold of 80% mortality. T0 to T6: = 0 to 6 months after spraying.

Results of cone bioassay show that both insecticide formulations sprayed were effective during six months of monitoring. Mortality will continue to be monitored until it is <80 percent for two consecutive months. We provided the details of mortality rates (24h to 120h) in supplementary file in Annex.

3.10 AIRBORNE FUMIGANT MORTALITY

Figure 14. Fumigant assay (24h and 120h mortality) of *An. gambiae* Kisumu and wild *An. gambiae* s.l. in Solenzo district sprayed with SumiShield WG

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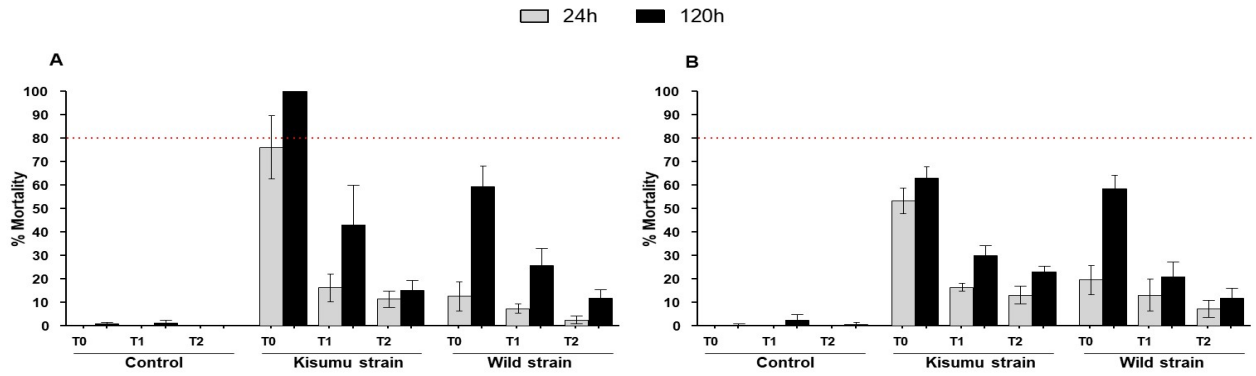
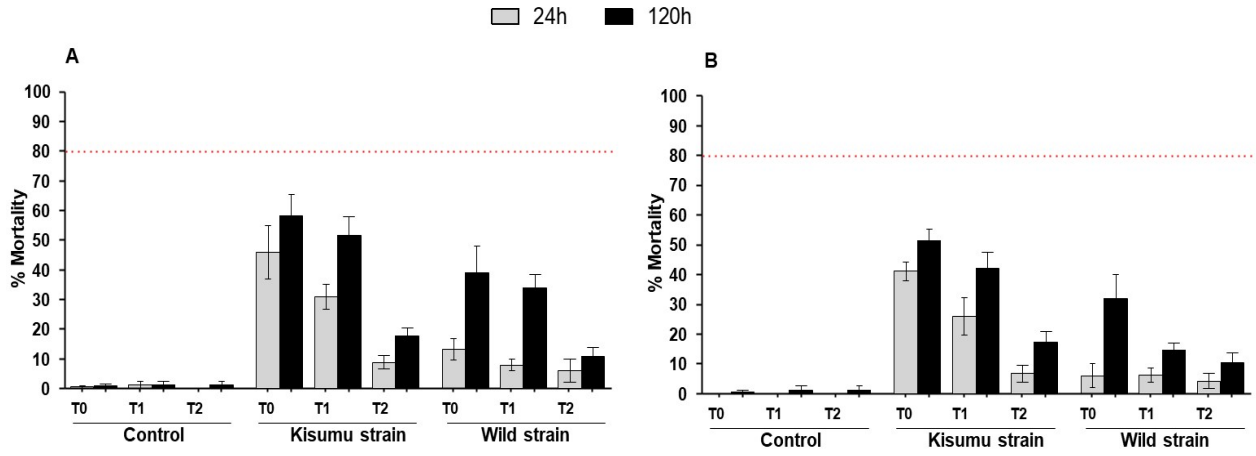


Note: Error bars represent the 95 percent confidence interval. Red dashed line represents the WHO threshold of 80 percent mortality. T0 to T2: = 0 to 2 months after spraying.

Figure 15. 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



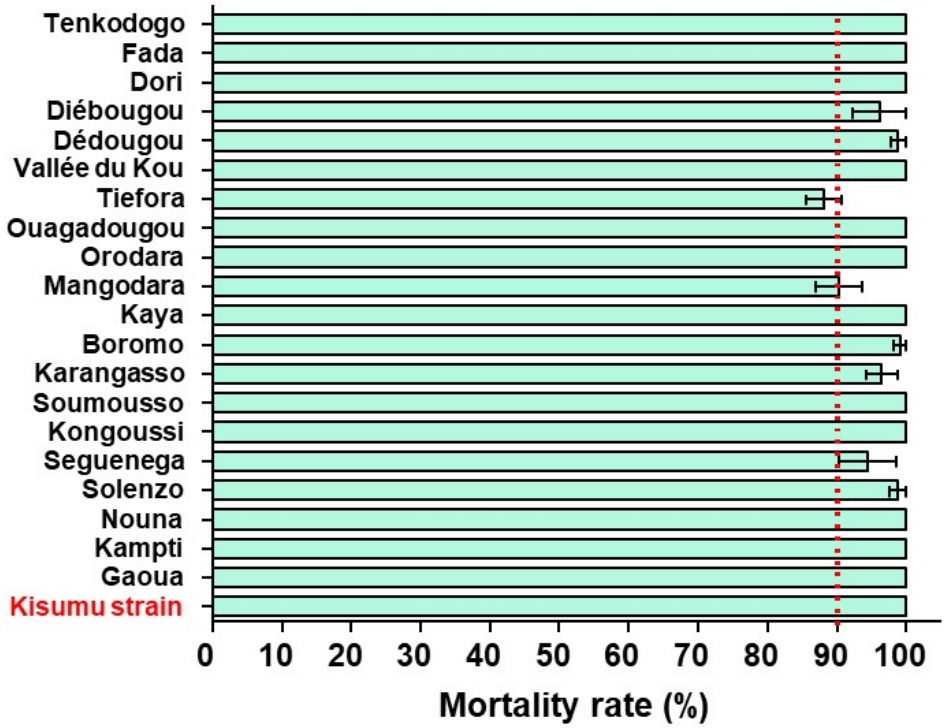
Note: Error bars represent the 95 percent confidence interval. Red dashed line represents the WHO threshold of 80 percent mortality. T0 to T2: = 0 to 2 months after spraying.

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.

3.11 INSECTICIDE SUSCEPTIBILITY DATA

Figure 16 summarizes the results of susceptibility tests performed with wild *An. gambiae* s.l. which were collected as larvae and reared to adults before being tested. Susceptibility (98-100 percent) was recorded 24 hours after exposure to 0.25% pirimiphos-methyl (PM) in 15 sites, including the two IRS sites of Solenzo and Kampti (Figure 16). There was possible resistance (90-98 percent mortality) recorded in Mangodara, Tiefora, Diebougou, Karangasso-Vigue and Seguenega (non-IRS sites).

Figure 16. Results of susceptibility tests with 0.25 percent pirimiphos-methyl in WHO tubes against wild *An. gambiae* s.l. from 20 sites



An. gambiae s.l. were resistant in all 20 sites to the three pyrethroid insecticides tested: alpha-cypermethrin 0.05%, deltamethrin 0.05% and permethrin 0.75%. Mortality rates were generally very low when exposed to the discriminating dose of alpha-cypermethrin, but when pre-exposed to PBO the mortality rate increased significantly in all 20 sites. Although there was generally a large increase in mortality with PBO pre-exposure, it usually did not fully restore susceptibility to alpha-cypermethrin and only produced mortality >90% in 6 sites (Diebougou, Ouagadougou, Mangodara, Karangasso-Vigue, Kampti and Gaoua) (Figure 17A). Trends were similar with deltamethrin, with PBO pre-exposure increasing mortality in all 20 sites (Figure 17B). Pre-exposure to PBO followed by permethrin also resulted in a significant increase in mortality in all 20 sites compared with permethrin alone. However, the increase in mortality was limited in several sites, with mortality <50% in 7 sites Vallée du Kou, Tiefora, Mangodara, Kongoussi, Solenzo, Nouna and Gaoua. In most sites there was at least one combination of PBO plus pyrethroid that resulted in high levels of mortality,

even if mortality wasn't fully restored. For example, in Gaoua only 30% mortality was reached with PBO plus permethrin, but PBO + deltamethrin or alpha-cypermethrin produced >90% mortality. However, in Fada, Dori, Vallée du Kou, Kongoussi and Seguenega, there were relatively low levels of mortality for PBO plus any of the three pyrethroids.

Of particular interest, are the results for Karangasso-Vigué and Soumousso, where PermaNet 3.0 ITNs (containing deltamethrin + PBO) were distributed in June 2019. Mortality with PBO + deltamethrin was 95 percent in Karangasso-Vigué, from 20 percent with deltamethrin alone and 96 percent in Soumousso, from 25 percent with deltamethrin alone. These results are extremely encouraging and indicate that PermaNet 3.0 would provide greater control in these sites than pyrethroid only ITNs. In conclusion, pre-exposure to PBO followed by alpha-cypermethrin, deltamethrin or permethrin resulted in substantial increases in mortality, reaching >80 percent in most sites indicating that metabolic-based resistance mechanisms played an important role in phenotypic resistance observed.

Figure 17. Results of synergist tests with (A) alpha-cypermethrin 0.05% and PBO 4% + alpha-cypermethrin 0.05%, (B) deltamethrin 0.05% and PBO 4% + deltamethrin 0.05% and (C) permethrin 0.75% and PBO 4% + permethrin 0.75%. Insecticide (black bars); PBO 4% + insecticide (grey bar)

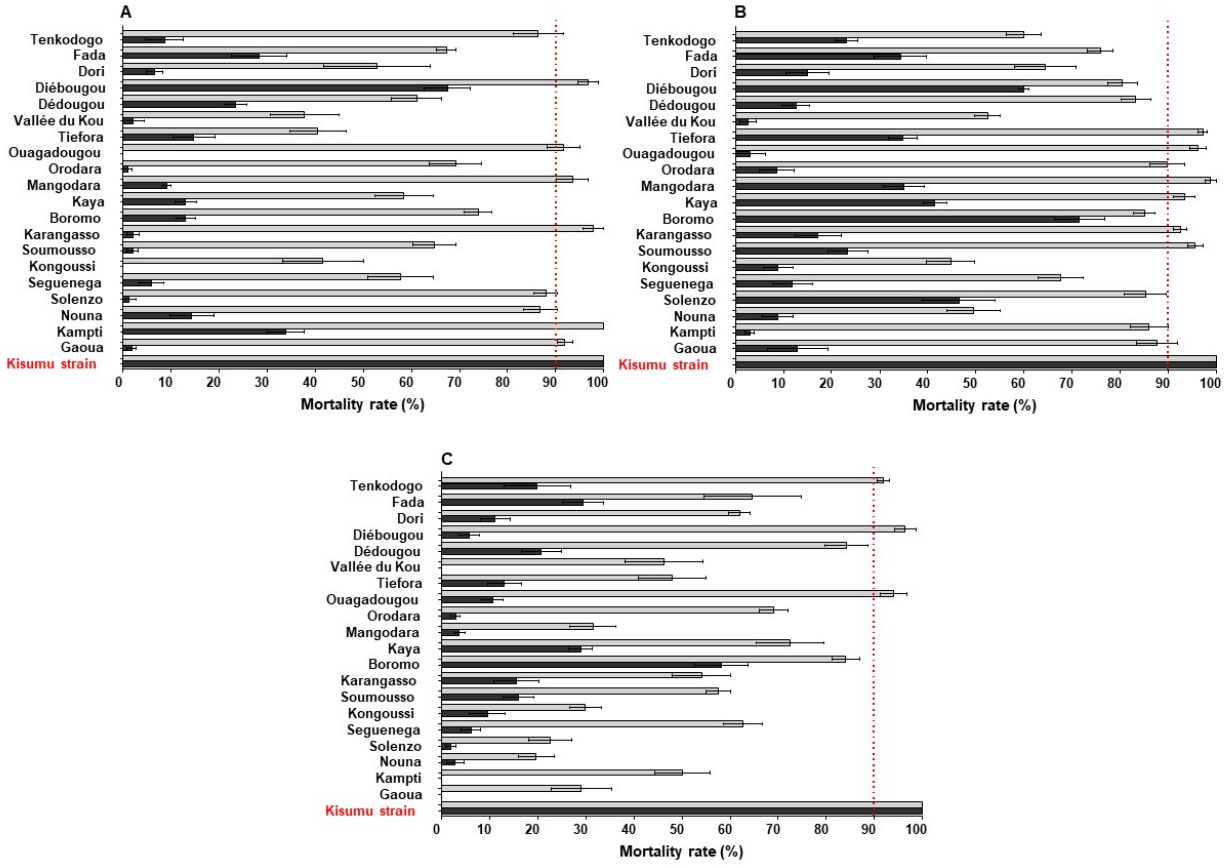


Figure 18 show the results of susceptibility tests performed on *An. gambiae* s.l. against chlorfenapyr using the interim diagnostic dose of 100µg/bottle, while figure 19 shows results for the 200 µg/bottle dose for sites where mortality was <100% with the 100µg/bottle dose. There was high mortality (98-100 percent) 24 hours after exposure with chlorfenapyr 100µg/bottle in most sites, except in Orodara and Nouna where

mortality was less than 90 percent. After 72 hours (the diagnostic time), results showed susceptibility to chlorfenapyr in all 14 sites (Figure 18). Testing with the 200µg/bottle dose in the 4 sites produced 100% mortality (Figure 19). These results indicated that wild *An. gambiae* s.l. are susceptible to chlorfenapyr in Burkina Faso.

Mortality rates was < 90% at 12 out of 20 sites after exposure with 2% clothianidin (Figure 20). However, after 120 hours (5 days), mortality was 100% at all the sites, including all three IRS sites (Figure 20). Therefore, SumiShield WG and Fludora Fusion WP-SB can continue to be used for IRS as part of a rotation strategy.

Figure 18. Results of susceptibility tests with chlorfenapyr 100µg/bottle in CDC bottle bioassays against *An. gambiae* s.l. in 14 sites. Mortality at 24h (orange bars) and mortality at 72h (green bar)

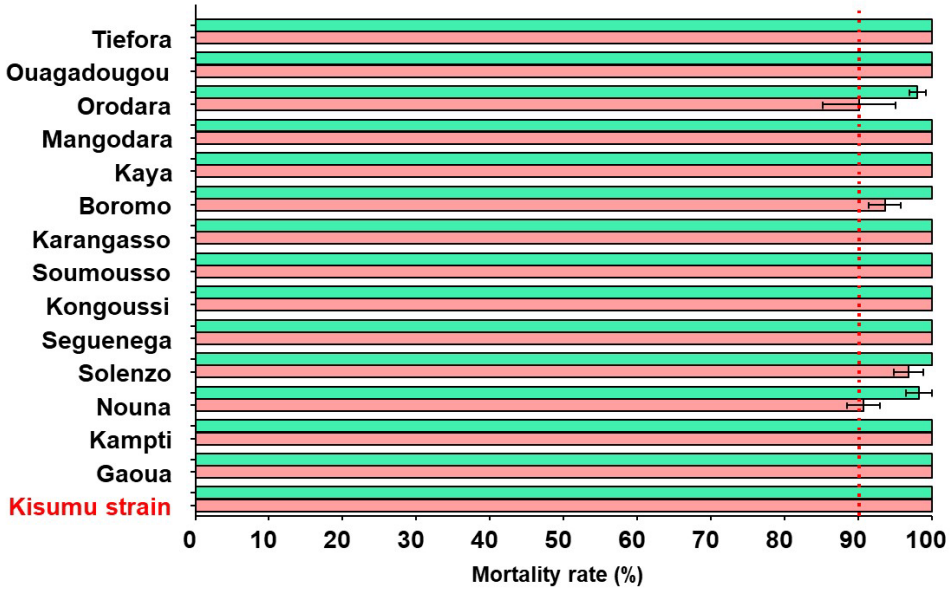


Figure 19. Results of susceptibility tests with chlorfenapyr 200µg/bottle in CDC bottle bioassays against *An. gambiae* s.l. in 4 sites. Mortality at 24h (orange bars) and mortality at 72h (green bar)

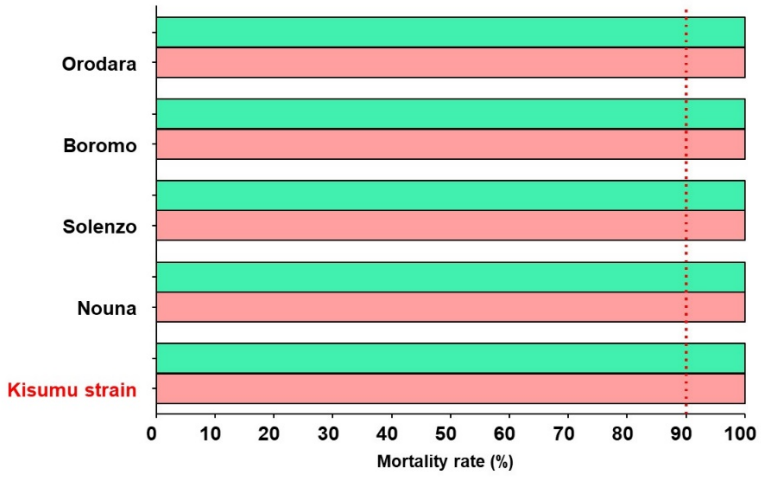
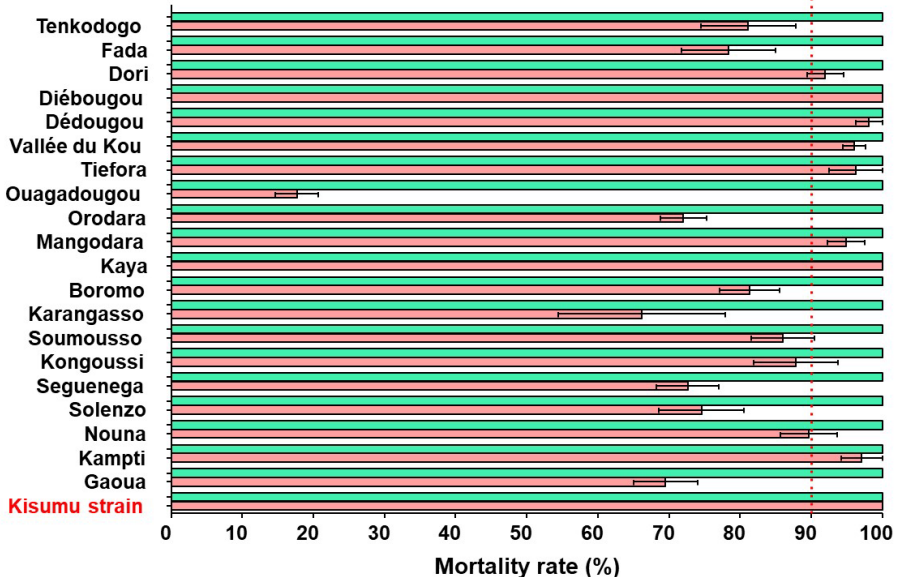


Figure 20. Results of susceptibility tests with 2% clothianidin in WHO tube tests against *An. gambiae* s.l. in 20 sites. Mortality at 24h (orange bars) and mortality at 5 days (green bar)



The results of resistance intensity tests for *An. gambiae* s.l. exposed in WHO test tubes to alpha-cypermethrin at 5x and 10x the diagnostic dose showed high resistance intensity in all sites (Table 5). The results showed high resistance intensity in 17 of 20 sites to deltamethrin, with moderate resistance intensity in three sites (Mangodara, Tiefora and Ouagadougou) (Table 6). The results were more variable for permethrin, with high resistance intensity in 12 sites and moderate intensity in 8 sites (Table 7).

Table 5. Mortality of *An. gambiae* s.l. after 24h post exposure to 5x and 10x concentrations of alphacypermethrin in WHO bioassays and status of resistance intensity

Sites	Alpha-cypermethrin diagnostic concentration (%)				Status
	5x (0.25%)		10x (0.5%)		
	n	% mortality [IC]	n	% mortality [IC]	
Kisumu strain	100	100	NA	NA	Susceptible
Gaoua	87	11.72 [1.89-21.54]	103	32.61 [14.63-50.58]	High
Kampti	98	28.41 [11.24-45.59]	106	37.58 [27.23-47.94]	High
Nouna	106	1.81 [0.00-5.16]	96	8.52 [0.00-18.57]	High
Solenzo	104	58.80 [47.77-69.83]	103	77.65 [69.82-85.49]	High
Seguenega	95	33.11 [22.75-43.48]	98	48.25 [37.02-59.49]	High
Kongoussi	104	38.32 [34.28-42.36]	95	43.18 [37.56-48.81]	High
Soumousso	104	69.56 [52.25-86.86]	101	85.14 [70.54-99.75]	High
Karangasso-V	93	44.67 [37.87-51.48]	87	62.19 [41.95-82.43]	High
Boromo	100	74.61 [52.27-96.94]	93	89.93 [71.39-100]	High
Kaya	94	48.54 [30.36-66.71]	96	58.41 [49.82-66.99]	High
Mangodara	97	66.02 [38.73-93.31]	95	92.43 [81.56-100]	High
Orodara	96	28.85 [7.41-50.28]	89	54.74 [36.98-72.51]	High
Ouagadougou	94	49.05 [35.86-62.24]	86	64.69 [48.33-81.05]	High
Tiéfora	96	31.26 [16.27-46.24]	98	71.36 [69.08-73.65]	High
Vallée du Kou	100	2.17 [0.00-9.09]	83	15.62 [13.50-17.74]	High
Dédougou	97	45.23 [24.67-65.79]	100	56.85 [40.49-73.21]	High
Diébougou	85	83.61 [75.19-92.02]	85	96.25 [88.63-100]	High
Dori	99	24.03 [11.39-36.68]	98	38.97 [30.57-47.38]	High
Fada N'Gourma	102	65.54 [57.37-73.72]	85	74.25 [56.90-91.60]	High
Tenkodogo	94	52.71 [35.67-69.75]	108	72.31 [62.76-81.85]	High

NA (Not applicable): 98–100 percent mortality at 5x dose indicates a low resistance intensity. Not necessary to assay at 10x dose. -: not tested

Table 6. Mortality of *An. gambiae* s.l. after 24h post exposure to 5x and 10x concentrations of deltamethrin in WHO bioassays and status of resistance intensity

Sites	Deltamethrin diagnostic concentration (%)				Status
	5x (0.25%)		10x (0.5%)		
	n	% mortality [IC]	n	% mortality [IC]	
Kisumu strain	100	100	NA	NA	Susceptible
Gaoua	90	28.02 [18.92-37.11]	109	77.84 [57.84-97.85]	High
Kampti	92	56.04 [38.07-74.01]	97	75.64 [67.29-83.99]	High
Nouna	108	35.30 [26.35-44.26]	96	62.81 [48.76-76.85]	High
Solenzo	97	71.93 [58.82-85.03]	105	93.36 [90.51-96.22]	High
Seguenega	92	55.18 [40.64-69.72]	101	76.79 [51.44-100]	High
Kongoussi	87	21.20 [10.49-31.92]	101	71.97 [56.46-87.49]	High
Soumousso	103	55.31 [51.51-59.12]	99	85.65 [70.31-100]	High
Karangasso-V	106	68.98 [54.11-83.85]	101	84.23 [74.40-94.06]	High
Boromo	108	84.15 [73.73-88.58]	107	88.89 [85.00-92.78]	High
Kaya	97	53.62 [45.94-61.30]	93	71.42 [59.65-83.18]	High
Mangodara	100	90.15 [82.40-97.90]	100	100	Moderate
Orodara	89	27.83 [8.28-47.36]	97	58.43 [44.07-72.80]	High
Ouagadougou	91	92.44 [80.58-100]	99	100	Moderate
Tiéfora	99	87.20 [70.30-100]	98	98.91 [95.45-100]	Moderate
Vallée du Kou	102	69.67 [62.87-76.46]	104	77.94 [69.55-86.33]	High
Dédougou	97	53.18 [31.63-74.72]	99	82.71 [68.61-96.80]	High
Diébougou	89	79.26 [63.75-94.77]	90	95.34 [89.15-100]	High
Dori	91	40.08 [26.66-53.50]	95	62.23 [48.70-75.75]	High
Fada N'Gourma	104	76.32 [65.47-87.16]	99	86.81 [80.26-93.36]	High
Tenkodogo	92	55.68 [32.36-79.00]	95	88.20 [78.90-97.51]	High

NA (Not applicable): 98–100 percent mortality at 5x dose indicates a low resistance intensity. Not necessary to assay at 10x dose.-: not tested

Table 7. Mortality of *An. gambiae* s.l. after 24h post exposure to 5x and 10x concentrations of permethrin in WHO bioassays and status of resistance intensity status

Sites	Permethrin diagnostic concentration (%)				Status
	5x (3.75%)		10x (7.5%)		
	n	% mortality [IC]	n	% mortality [IC]	
Kisumu strain	100	100.0	NA	NA	Susceptible
Gaoua	99	48.92 [35.574-62.27]	106	96.11 [90.91-100]	High
Kampti	87	76.61 [61.03-92.19]	95	100	Moderate
Nouna	103	41.67 [22.97-60.38]	102	82.41 [67.82-96.99]	High
Solenzo	103	77.84 [45.49-100]	96	96.91 [90.76-100]	High
Seguenega	105	65.70 [51.79-79.62]	103	92.65 [82.28-100]	High
Kongoussi	95	31.52 [16.08-46.96]	85	67.06 [49.67-84.45]	High
Soumousso	102	95.39 [90.01-100]	104	98.96 [95.64-100]	Moderate
Karangasso-V	90	93.87 [82.06-100]	103	100	Moderate

Sites	Permethrin diagnostic concentration (%)				Status
	5x (3.75%)		10x (7.5%)		
	n	% mortality [IC]	n	% mortality [IC]	
Boromo	92	95.69 [90.03-100]	98	100	Moderate
Kaya	98	68.69 [48.96-88.42]	98	89.91 [79.09-100]	High
Mangodara	100	82.72 [68.39-97.05]	97	98.86 [95.25-100]	Moderate
Orodara	91	77.85 [45.74-100]	90	97.73 [90.49-100]	High
Ouagadougou	104	10.54 [3.21-17.87]	103	93.98 [87.38-100]	High
Tiéfora	98	84.59 [77.77-91.40]	99	95.05 [85.89-100]	High
Vallée du Kou	100	46.66 [33.48-59.83]	103	88.99 [76.89-100]	High
Dédougou	101	89.20 [79.41-99.00]	103	97.15 [91.46-100]	High
Diébougou	80	88.29 [73.94-100]	95	100	Moderate
Dori	93	81.79 [73.88-89.70]	105	99.04 [95.98-100]	Moderate
Fada N'Gourma	105	91.32 [77.40-100]	103	98.03 [94.40-100]	Moderate
Tenkodogo	93	86.01 [74.74-97.29]	96	95.76 [90.08-100]	High

NA (Not applicable): 98–100 percent mortality at 5x dose indicates a low resistance intensity. Not necessary to assay at 10x dose. -: not tested.

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

The allele 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 and 98 percent. It was generally at moderate frequency in the Centre West and North with lowest frequency reported in Kaya and Segeuenga at less than 40 percent. In *An. coluzzi* populations the allele frequency of the West African kdr-L1014F mutation did not reach 80 percent in any sites. This mutation was also present within *An. arabiensis* populations mainly from the Southwest in Gaoua and Soumousso being extended to the central sites (Ouagadougou).

The kdr-L1014S mutation was also found in *An. gambiae* populations, mostly in Soumousso (in West) and Seguenega (North). It was found in moderate frequencies within *An. coluzzii* populations and was exceptionally high in Kampti and Gaoua. This mutation was found at relatively high frequency in *An. arabiensis* particularly in the Southwest where it reached 60 percent in Kampti and Gaoua. The two mutations occurred simultaneously within the same populations of *An. gambiae* s.l., indicating the occurrence of multiple resistance mechanisms (L1014F, L1014S and metabolic resistance).

The *ace-1R* G119S mutation was reported both in *An. gambiae* and *An. coluzzii* populations at increased frequencies in most sites tested in Western Burkina Faso, although the allele frequency did not reach 50 percent (Annex 10). The *Ace-1R* mutation can cause resistance to organophosphates and carbamates; therefore, it should be closely monitored in areas where IRS with pirimiphos-methyl (an organophosphate) is conducted.

4. CONCLUSIONS

Fludora Fusion WP-SB and SumiShield WG lasted for at least seven months in all sites (cone bioassay ongoing). Both formulations lasted long enough to cover the entire high transmission season in Burkina Faso. The airborne fumigant effect was initially highly effective for the two formulations, but after three months, there was minimal fumigant effect.

There was 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 sub-locations compared to unsprayed areas, but not in rural sites where biting rates were higher. This is probably due to the high densities before the intervention and occurrence of suitable breeding sites in those settings. The density of *An. gambiae* s.l. collected from PSC was slightly lower in IRS sites compared to their paired unsprayed control sites, particularly in Kampti.

There was no apparent reduction in parity rates of *An. gambiae* s.l. except in Kongoussi (which was no longer an IRS site). Conversely in Nouna the parity rate was lower than that of Solenzo (sprayed).

The sporozoite rates, one of the most relevant entomological parameters was generally lower in IRS sites than unsprayed sites. However, while the sporozoite rate was lower in sprayed Kampti than in unsprayed Gaoua it was still extremely high. The sporozoite rates were highest in the south-west and were considerably lower in the more northern sites such as Kongoussi and Seguenega (despite both being unsprayed).

IRS appears to have had a small impact on the entomological inoculation rate in 2020 compared to unsprayed control sites. However, the high HBR from rural sites combined with small impact on sporozoite rate meant the impact of IRS was small and the EIR in sprayed sites remained high. In the two sites where PBO ITNs were distributed, the EIR was similar indoors averaging 90 i/b/p in Soumousso and 110 i/b/p in Karangasso-Vigue. We have no pre-distribution data to determine the impact of PBO nets. However, despite the use of PBO nets the EIR still remained high, meaning that more interventions are needed. Additional vector control measures could include housing modification, potentially through the use of insecticide treated eave tubes to limit entry of malaria vectors and provide additional control of malaria vectors, although more supporting data is needed to determine the cost-effectiveness of this approach.

Insecticide susceptibility tests revealed that *An. gambiae* s.l. were resistant to all pyrethroids tested, but pre-exposure to PBO increased mosquito susceptibility to alpha-cypermethrin, deltamethrin and permethrin in 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 moderate or high for permethrin. This highlights that high resistance intensity throughout Burkina Faso may be undermining the performance of pyrethroid only ITNs. On a positive note, susceptibility to

chlorfenapyr was recorded at all sites. These results support the distribution of PBO and chlorfenapyr ITNs in Burkina Faso instead of pyrethroid only ITNs.

Susceptibility data of insecticides used for IRS showed that there was full susceptibility of *An. gambiae* s.l. to clothianidin observed at all sites tested, including both IRS locations. While susceptibility to pirimiphos-methyl was also recorded in both IRS sites and most sites nationwide. Therefore, insecticide formulations containing pirimiphos-methyl and clothianidin can continue to be used in Burkina Faso for IRS in rotation.

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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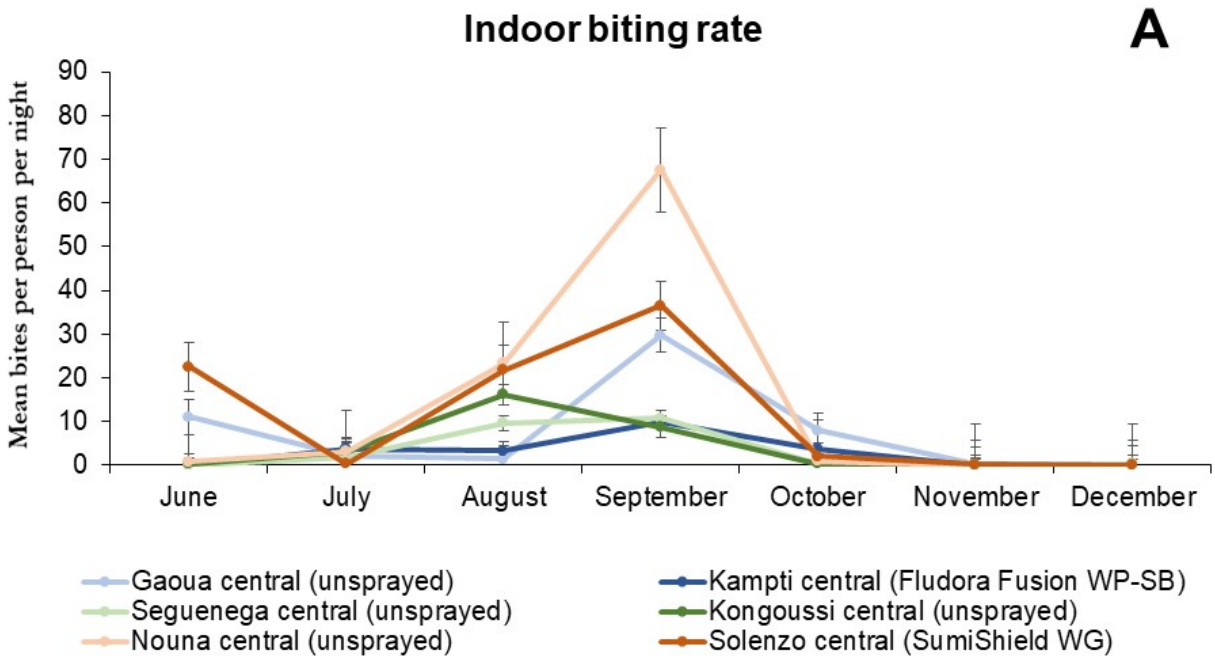
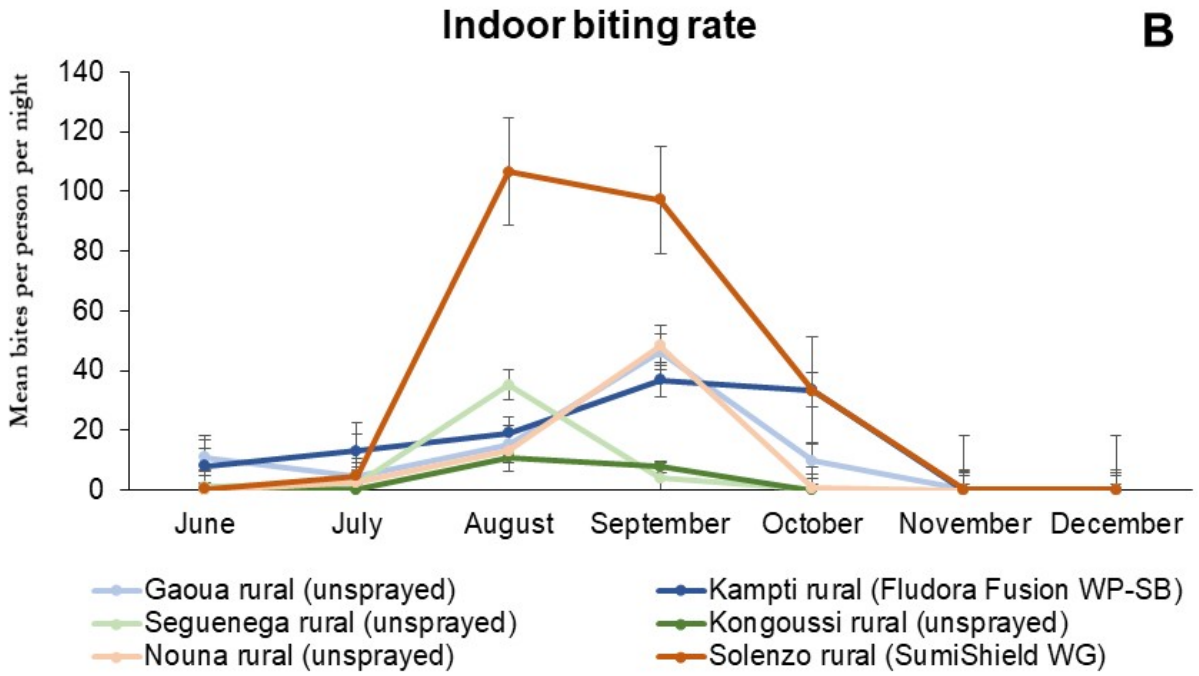
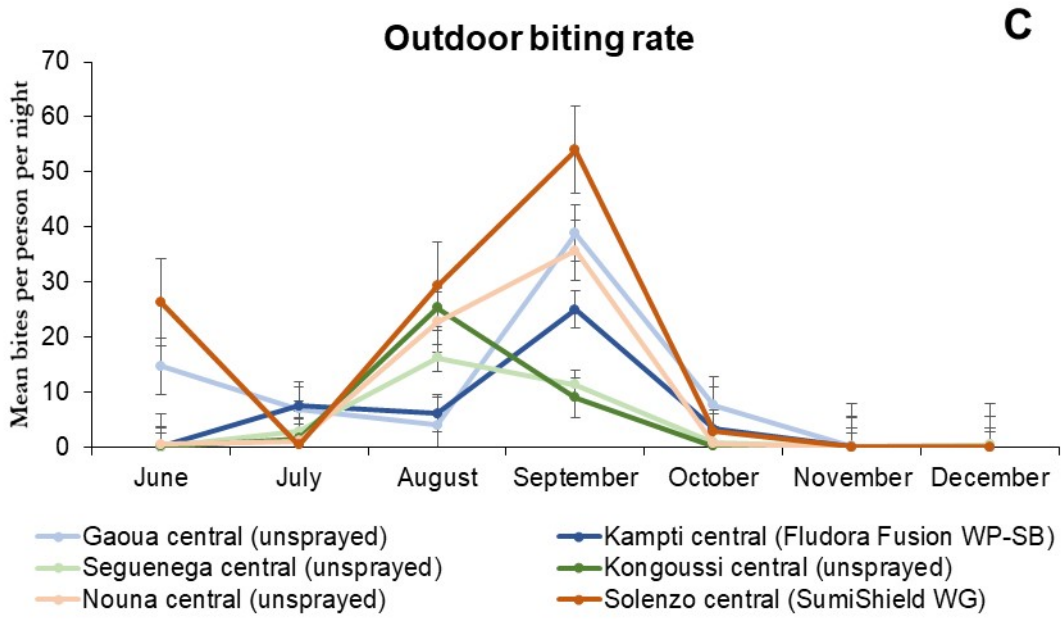
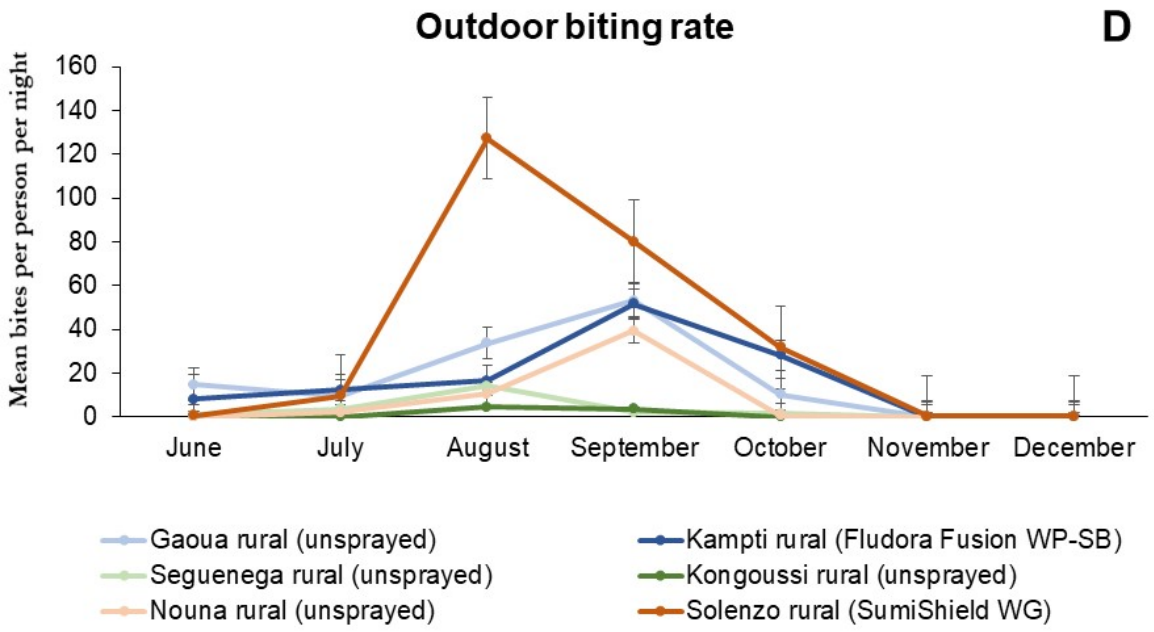
ANNEX

Annex 1. Nightly biting rate of *An. gambiae* s.l. collected by HLC in IRS and unsprayed sites indoor (A& B) and outdoor (C&D) from June to December 2020




Indoor biting rate
B
Mean bites per person per night
140
120
100
80
60
40
20
0
June
July
August
September
October
November
December
Gaoua rural (unsprayed)
Kampti rural (Fludora Fusion WP-SB)
Seguenega rural (unsprayed)
Kongoussi rural (unsprayed)
Nouna rural (unsprayed)
Solenzo rural (SumiShield WG)


Outdoor biting rate
C
Mean bites per person per night
70
60
50
40
30
20
10
0
June
July
August
September
October
November
December
Gaoua central (unsprayed)
Kampti central (Fludora Fusion WP-SB)
Seguenega central (unsprayed)
Kongoussi central (unsprayed)
Nouna central (unsprayed)
Solenzo central (SumiShield WG)


Outdoor biting rate
D
Mean bites per person per night
160
140
120
100
80
60
40
20
0
June
July
August
September
October
November
December
Gaoua rural (unsprayed)
Kampti rural (Fludora Fusion WP-SB)
Seguenega rural (unsprayed)
Kongoussi rural (unsprayed)
Nouna rural (unsprayed)
Solenzo rural (SumiShield WG)

Annex 2. Mean infection rate of *An. gambiae* s.l. collected by HLC (indoor + outdoor) collection from June to December 2020 in the IRS and unsprayed central and rural sites

Infection rate of *An. gambiae* s.l. from central sites						
Months	**Gaoua central (Unsprayed)** (N=283)	**Kampti central Fludora® Fusion WP-SB** (N=178)	**Nouna central (Unsprayed)** (N=196)	**Solenzo central Shumishield WG** (N=163)	**Seguenega central (Unsprayed)** (N=201)	**Kongoussi central (unsprayed)** (N=191)
June	6 (3/50)	0 (0/3)	0 (0/7)	0 (0/3)	0 (0/26)	3.3 (1/33)
July	33.33 (13/39)	6 (3/50	4 (2/50)	0 (0/7)	4 (2/50)	0 (0/50)
August	20 (10/50)	10 (3/30)	6 (3/50)	0 (0/50)	2 (1/50)	4 (2/50)
September	2 (1/50)	10.2 (5/49)	2 (1/50)	4 (2/50)	8 (4/50)	2 (1/50)
October	34 (17/50)	33.33 (15/45)	50 (18/36)	22.44 (11/49)	13.33 (2/15)	0 (0/6)
November	16.16 (1/6)	0 (0/1)	33.33 (1/3)	0 (0)	25 (1/4)	0 (0)
December	15.78 (6/38)	0 (0)	0 (0)	0 (0/4)	0 (0/6)	0 (0/2)
Mean infection rate rate % (P/N)	**18.02 (51/283)**	**14.6 (26/178)**	**12.75 (25/196)**	**7.97 (13/163)**	**4.97 (10/201)**	**2.09 (4/191)**
Infection rate of *An. gambiae* s.l. from rural sites						
Months	**Gaoua rural (Unsprayed)** (N=265)	**Kampti rural Fludora® Fusion WP-SB** (N=241)	**Nouna rural (Unsprayed)** (N=194)	**Solenzo rural Sumishield WG** (N=228)	**Seguenega rural (Unsprayed)** (N=174)	**Kongoussi rural (unsprayed)** (N=164)
June	18 (9/50)	10,52 (4/38)	0 (0)	0 (0/19)	0 (0/20)	0 (0/11)
July	36 (18/50)	26 (13/50)	2 (1/50)	2 (1/50)	5 (5/50)	4,34 (2/46)
August	52 (26/50)	26 (13/50)	2 (1/50)	4 (2/50)	2 (2/50)	4,08 (2/49)
September	16 (8/50)	6 (3/50)	0 (0/50)	8 (4/50)	5 (5/50)	6 (3/50)
October	46 (23/50)	48 (24/50)	33,33 (11/33)	6 (3/50)	1 (1/4)	0 (0/5)
November	50 (2/4)	33,33 (1/3)	27,27 (3/11)	25 (1/4)	0 (0)	50 (1/2)
December	18.18 (2/11)	0 (0)	0 (0)	20 (1/5)	0 (0)	100 (/1)
Mean infection rate % (P/N)	**33.20 (88/265)**	**24.06 (58/241)**	**8.24 (16/194)**	**5.26 (12/228)**	**7.47 (13/174)**	**5.48 (9/164)**

Annex 3. Infection rates of *An. gambiae* s.l. females to *P. falciparum* from indoors and outdoors collections in PBO-ITNs sites in 2020

Sporozoite rate of *An. gambiae* s.l. from HLC indoor+outdoors collections		
Months	**Karangasso-Vigué**	**Soumousso**
June	0 (0/50)	0 (0/50)
July	28 (14/50)	18 (9/50)
August	2 (1/50)	6 (3/50)
September	6 (3/50)	6 (3/50)
October	14 (7/50)	14 (7/27)
November	12.5 (2/16)	26.31 (5/19)
December	20 (1/5)	25 (1/4)
Total	**10.33% (28/271)**	**10.25% (28/273)**

Annex 4. Nightly entomological inoculation rate of *An. gambiae s.l.* collected by HLC (indoor + outdoor) collection from June to December 2020 in the IRS and unsprayed central 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 (Unsprayed)**
June	0.77	0.00	0.00	0.00	0.00	0.00
July	1.52	0.34	0.10	0.00	0.09	0.00
August	0.59	0.49	0.77	0.00	0.46	1.02
September	0.66	1.78	0.22	0.33	4.13	0.91
October	2.66	1.17	0.44	0.06	0.12	0.00
November	0.02	0.00	0.00	0.00	0.00	0.00
December	0.06	0.00	0.00	0.00	0.00	0.00
Mean EIR per night	**1.59**	**0.65**	**0.50**	**0.37**	**0.56**	**0.22**
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 (Unsprayed)**
June	1.52	0.86	0.00	0.00	0.00	0.00
July	2.54	3.30	0.05	0.31	0.14	0.02
August	12.74	4.62	0.24	4.68	0.49	0.31
September	7.94	2.65	0.00	7.10	0.16	0.34
October	3.31	15.69	0.23	1.98	0.00	0.00
November	0.03	0.00	0.00	0.08	0.00	0.00
December	0.00	0.00	0.00	0.04	0.00	0.00
Mean EIR per night	**4.59**	**3.96**	**0.69**	**1.92**	**0.34**	**0.11**

Annex 5. Monthly entomological inoculation rate of *An. gambiae* s.l. collected by HLC (indoor + outdoor) collection from June to December 2020 in the IRS and unsprayed central 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	23.18	0.00	0.00	0.00	0.00	0.12
July	45.62	10.13	2.85	0.00	2.63	0.00
August	17.63	14.63	23.06	0.00	13.80	30.60
September	19.88	53.36	6.64	9.98	123.90	27.15
October	79.69	35.00	13.13	1.68	3.75	0.00
November	0.61	0.00	0.00	0.00	0.00	0.00
December	1.78	0.00	0.00	0.00	0.00	0.00
Mean EIR per month	**47.74**	**19.67**	**15.10**	**11.02**	**16.72**	**6.61**
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	45.56	25.84	0.00	0.00	0.00	0.00
July	76.28	98.96	1.39	9.34	4.31	0.49
August	382.20	138.45	7.05	140.25	14.78	9.41
September	238.20	79.54	0.00	213.00	4.78	10.24
October	99.19	470.70	6.87	59.40	0.04	0.00
November	0.94	0.00	0.00	2.34	0.00	0.00
December	0.00	0.00	0.00	1.13	0.00	0.00
Mean EIR per month	**137.84**	**118.95**	**20.70**	**57.51**	**10.26**	**3.27**

Annex 6. Monthly entomological inoculation rate of *An. gambiae* s.l. collected by HLC indoor and outdoor collection from June to December 2020 in PBO-ITNs sites

EIR per month of *An. gambiae* s.l. indoor and outdoor		
Months	**Karangasso-Vigué**	**Soumousso**
June	0.00	0.00
July	10.50	27.00
August	2.93	9.45
September	47.03	31.50
October	19.95	12.60
November	0.47	0.00
December	1.50	0.00
Overall EIR (indoor + outdoor) for 7 months	**82.37**	**80.55**

Annex 7. 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

			Genotypes					Genotypes		
Species	Sites	N	1014L 1014L	1014L 1014F	1014F 1014F	f(L1014F)	1014L 1014L	1014L 1014S	1014S 1014S	f(1014S)
An. arabiensis	**Kampti**	13	1	5	7	**0.731**	11	2	0	**0.077**
	Gaoua	5	2	1	2	**0.500**	5	0	0	**0.000**
	Solenzo	2	0	2	0	**0.500**	0	2	0	**0.500**
	Nouna	1	1	0	0	**0.000**	1	0	0	**0.000**
	Kongoussi	0	NA	NA	NA	**NA**	NA	NA	NA	**NA**
	Seguenega	10	3	6	1	**0.400**	8	2	0	**0.100**
	Orodara	3	0	1	2	**0.833**	2	1	0	**0.167**
	Soumousso	11	0	2	9	**0.909**	10	1	0	**0.045**
	Ouagadougou	42	0	10	32	**0.881**	36	6	0	**0.071**
	Boromo	21	8	13	0	**0.310**	21	0	0	**0.000**
	Mangodara	1	0	0	1	**1.000**	1	0	0	**0.000**
	Kaya	10	6	2	2	**0.300**	9	1	0	**0.050**
An. coluzzii	**Kampti**	2	0	1	1	**0.750**	1	1	0	**0.250**
	Gaoua	3	1	2	0	**0.333**	2	1	0	**0.167**
	Solenzo	38	9	15	14	**0.566**	28	10	0	**0.132**
	Nouna	40	5	25	10	**0.562**	32	8	0	**0.100**
	Kongoussi	48	17	23	8	**0.406**	35	13	0	**0.135**
	Seguenega	12	4	7	1	**0.375**	9	3	0	**0.125**
	Orodara	5	1	1	3	**0.700**	4	1	0	**0.100**
	Soumousso	2	0	1	1	**0.750**	1	1	0	**0.250**
	Ouagadougou	7	0	6	1	**0.571**	5	2	0	**0.143**
	Boromo	8	2	2	4	**0.625**	8	0	0	**0.000**
	Mangodara	1	1	0	0	**0.000**	1	0	0	**0.000**
	Kaya	9	6	3	0	**0.167**	8	1	0	**0.056**
An. gambiae s.s	**Kampti**	35	1	3	31	**0.929**	33	2	0	**0.029**
	Gaoua	42	2	9	31	**0.845**	42	0	0	**0.000**
	Solenzo	10	1	2	7	**0.800**	9	1	0	**0.050**
	Nouna	9	0	6	3	**0.667**	7	2	0	**0.111**
	Kongoussi	2	0	2	0	**0.500**	0	2	0	**0.500**
	Seguenega	28	1	16	11	**0.679**	21	7	0	**0.125**
	Orodara	42	0	5	37	**0.940**	41	1	0	**0.012**
	Soumousso	37	11	16	10	**0.486**	28	9	0	**0.122**
	Ouagadougou	1	0	0	1	**1.000**	1	0	0	**0.000**

Species	Sites	N	Genotypes					Genotypes		
			1014L 1014L	1014L 1014F	1014F 1014F	f(L1014F)	1014L 1014L	1014L 1014S	1014S 1014S	f(1014S)
	Boromo	21	4	11	6	**0.548**	20	1	0	**0.024**
	Mangodara	48	2	3	43	**0.927**	47	1	0	**0.010**
	Kaya	27	10	11	6	**0.426**	26	1	0	**0.019**

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

			Genotypes			
Species	Sites	N	119G 119G	119G 119S	119S 119S	f(119S)
An. arabiensis	**Kampti**	13	13	0	0	0.000
	Gaoua	9	9	0	0	0.000
	Solenzo	3	3	0	0	0.000
	Nouna	NA	NA	NA	NA	NA
	Kongoussi	NA	NA	NA	NA	NA
	Seguenega	5	5	0	0	0.000
	Orodara	4	4	0	0	0.000
	Soumousso	35	35	0	0	0.000
	Ouagadougou	45	44	1	0	0.011
	Boromo	2	2	0	0	0.000
	Mangodara	1	1	0	0	0.000
	Kaya	1	1	0	0	0.000
An. coluzzii	**Kampti**	NA	NA	NA	NA	NA
	Gaoua	1	1	0	0	0.000
	Solenzo	35	32	3	0	0.043
	Nouna	2	2	0	0	0.000
	Kongoussi	25	25	0	0	0.000
	Seguenega	22	22	0	0	0.000
	Orodara	5	5	0	0	0.000
	Soumousso	6	6	0	0	0.000
	Ouagadougou	NA	NA	NA	NA	NA
	Boromo	13	11	2	0	0.077
	Mangodara	NA	NA	NA	NA	NA
	Kaya	25	24	1	0	0.020
An. gambiae s.s	**Kampti**	37	31	6	0	0.081
	Gaoua	31	23	7	1	0.145
	Solenzo	11	7	4	0	0.182
	Nouna	41	40	1	0	0.012
	Kongoussi	23	22	1	0	0.022
	Seguenega	22	22	0	0	0.000
	Orodara	39	21	18	0	0.231
	Soumousso	7	5	2	0	0.143
	Ouagadougou	2	2	0	0	0.000
	Boromo	32	31	1	0	0.016
	Mangodara	46	27	19	0	0.207
	Kaya	23	22	1	0	0.022

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